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Patentanmeldung Nr.

Patent application No. Demande de brevet nº

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Si aucun titre n'est indiqué se referer à la description.)

Method for hla typing

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#### Method for HLA typing

The present invention relates to a method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) at a given position simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primers) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases. This method is particularly well suited for DNA-based HLA typing and in combination with a suitable selection of sites tested, it is superior in ease of operation to conventional HLA typing methods.

The most important of the genome projects, the complete sequence of the human genome, is finished. This project reveals the complete sequence of the 3 billion bases and the relative positions of all estimated 30.000 genes in this genome. Having this sequence opens unlimited possibilities for the elucidation of gene function and interaction of different genes. In recent years a systematic effort (SNP consortium) has been underway to identify single nucleotide polymorphisms (SNPs) throughout the human genome and so far several million of these differences between different human beings have been identified (dbSNP contained 5.5 million SNPs in October 2003).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI) has revolutionized the mass spectrometric analysis of biomolecules (Karas, M. & Hillenkamp, F. Anal. Chem. 60, 2299-2301 (1988)). The field of DNA analysis by mass spectrometry was recently extensively reviewed by Tost and Gut (Mass Spectrometry Reviews, 21, 388-418 (2002)) and Sauer and Gut (Journal of Chromatography B, 782, 73-87, (2002)). MALDI has been applied to the analysis of DNA in variations that range from the analysis of PCR products to approaches using allele-specific termination to single nucleotide primer extension reactions and sequencing (Liu, Y.-H., et al. Rapid Commun. Mass Spectrom. 9, 735-743 (1995);





Ch'ang, L.-Y., et al. Rapid Commun. Mass Spectrom. 9, 772-774 (1995); Little, D.P., et al. J. Mol. Med. 75, 745-750 (1997); Haff, L. & Smirnov, I.P. Genome Res. 7, 378-388 (1997), Fei, Z., Ono, T. & Smith, L.M. Nucleic Acids Res. 26, 2827-2828 (1998); Ross, P., Hall, L., Smirnov, I. & Haff, L. Nature Biotech. 16, 1347-1351 (1998); Ross, P.L., Lee, K. & Belgrader, P. Anal. Chem. 69, 4197-4202 (1997); Griffin, T.J., Tang, W. & Smith, L.M. Nature Biotech. 15, 1368-1372 (1997); Köster, H., Higgins, G.S & Little, D.P. US Patent 6,043,031). These methods are used to genotype previously identified mutations, SNPs, or insertion/deletions (indels). Spin column purification and/or magnetic bead technology, reversed-phase purification, or ion-exchange resins are frequently applied prior to mass spectrometric analysis.

The GOOD assay (IG Gut et S. Beck: US 6,268,812; IG Gut et al: US 6,503,710) is a method for SNP genotyping that uses MALDI mass spectrometry for detection (Sauer et al. 28, e13 and e100 (2000)). Allele-distinction is based on primer extension. In order to make products more amenable to MALDI analysis a substantial part of the primer is removed prior to mass spectrometric analysis. A further element that is included is charge tagging. This means that the final product is conditioned such that it carries either a single positive or a single negative charge. Generally this is achieved by alkylation of a phosphorothicate backbone and in some instances including a quaternary ammonium group to the penultimate base of the primer. The attachment of the quaternary ammonium group gives options for the design of multiplexes - individual SNPs can be moved up or down in the mass spectrum to achieve optimal resolution and separation.

The major histocompatibility complex (MHC) of humans is a cluster of genes on chromosome 6p21. It is of greatest importance as many diseases show association with genes in this region of the genome. All human leukocyte antigen (HLA) coding genes are found in the MHC. The HLA genes are highly variable and implicated in tissue transplantation, immunity and autoimmune disease such as diabetes, psoriasis, lupus, Crohn's disease, colitis, arthritis, and others. The HLA class I genes are HLA-A, HLA-B, HLA-C, ..... The HLA class II genes are HLA-DR, HLA-DQ, HLA-DP,....





HLA typing methods differ dramatically in their approaches. Serological tests can be carried out but have only limited resolution. In the last 15 years the DNA sequence of the MHC has been extensively studied and high resolution typing now makes use of a wealth of DNA sequence information. Methods for DNA based HLA typing range from SSA (sequence specific amplification) where combinations of primers that are specific for different alleles are used to carry out PCR (US 5,545,526). Primers are combined in a way that the sizing of the PCR products allows unambiguous assignment of present base combinations. Multiple combinations are used to identify HLA types. The procedure works its way through a tree of combinations starting with a grouping into rough classes from where on further tests are carried out with specific reagents to subdivide in a class. This method is also known as SSP (sequence specific primers). An alternative method is termed SSOP (sequence specific oligonucleotide probes; US 6,503,707). Here a locus specific PCR is carried out followed by hybridisation with sequence specific oligonucleotide probes. As sequencing technology (and in particular the software for sequence calling) has dramatically improved over the last decade it now is also possible to gain a good degree of identification of HLA types by sequencing (WO 98/35059). Effectively a locus-specific PCR product is sequenced. Problems that arise here are that heterozygous individuals occasionally give rise to ambiguous haplotype calls that can not be resolved (Robinson, J.; Waller, M.J.; Marsh, St.G.E.: "Exon Identities and Ambiguous Typing Combinations"; IMGT/HLA Database; October 2003). The inclusion of allele-specific PCR helps achieve certainty. Resolution requires multiple products per locus to be generated and sequenced. However, as sequencing results can be very convoluted the interpretation in absence of allele-specific PCR can be cumbersome. All together the sequence-based typing requires many iterations in application. Reference strand mediated conformation analysis (RSCA) is a method used to study samples that potentially have a previously unknown sequence in their HLA (Correl et al., Tissue Antigens 56, 82-86, 2000). For a recent review for the reasoning of HLA typing as well as methodological advances see Petersdorf et al. (Tissue Antigens, 61, 1-11, 2003).



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The object of the present invention is a method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primer pool) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases.

#### In the present invention:

- "HLA" means the human leukocyte antigen locus on chromosome 6p21, consisting of HLA genes (HLA-A, HLA-B, HLA-C, HLA-DRB1,...) that are used to determine the degree of matching, for example, between a recipient and a donor of a tissue graft.
  - "HLA typing" means the identification of a known HLA allele of a given locus (HLA-A, HLA-B, HLA-C, HLA-DRB1,...).
- "HLA allele" means a nucleotide sequence within a locus on one of the two parental chromosomes.
  - "HLA-A" means the DNA sequence of exons 2 and 3 of the HLA-A gene.
  - "HLA-B" means the DNA sequence of exons 2 and 3 of the HLA-B gene.
  - "HLA-DRB1" means the DNA sequence of exon 2 of the HLA-DRB1 gene.
- "Polymorphism" means individual positions in a DNA sequence that exist in different variants.
  - "Haplotype" means the DNA sequence of one of the two alleles in a give region of the genome.



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- "Mini-haplotype" means 2-6 contiguous bases on one parental allele.
- "Primer pools" or "pools of primers" means sets of primers that are used in one primer extension reaction. For each known HLA allele at least one primer is in the pool that is completely complementary in sequence. This assures perfect annealing. Mismatches that are more than 4 bases from the 3'end of the primer do not affect the results of the GOOD assay, as all of those bases are removed by 5'phosphodiesterase after the primer extension reaction. Primers of the pool containing mismatches in the last few bases are not extended by the DNA polymerase and thus not observable.
- "MALDI mass spectrometer" means a mass spectrometer that uses matrixassisted laser desorption/ionization for the volatilisation of a sample and time-offlight analysis for mass separation.
  - "Subgroup" means alleles, which are identical after the mini-haplotyping of the first set of selected positions. For the high resolution typing we resolve subgroups generated with 10 mini-haplotyping reactions. The criteria for resolving subgroups are: a) they still contain alleles with different two-digit types, b) subgroups with more than four alleles, and c) subgroups with frequent alleles (see list below).
- Here we show a methodology for the determination of sequence motifs of 2-6 bases 20 in very polymorphic regions of genomes. In principle this methods equates to the determination of mini-haplotypes of 2-6 bases. The individual parental minihaplotypes can be determined in one reaction without ambiguities. This methodology is applied to a chosen set of positions for HLA typing of HLA-A, HLA-B, and HLA-DRB1. The sets disclosed here have different purposes. First sets 25 of 19, 19, and 10 positions are suggested to distinguish a maximum of HLA alleles in HLA-A, HLA-B, and HLA-DRB1, respectively, with respect to differentiating alleles that are frequent in the general population from ones that are rare. The frequent alleles that were screened for are A\*0101, A\*0201, A\*0301, A\*2301, A\*2402, A\*2902, A\*3001 and A\*3002 for HLA-A, B\*0702, B\*0801, B\*1302, 30 B\*1501, B\*1801, B\*3501, B\*3503, B\*4001, B\*4402, B\*4403, B\*5101 and B\*5701 for HLA-B, and DRB1\*0101, DRB1\*0301, DRB1\*0401, DRB1\*0701,





DRB1\*1101, DRB1\*1104, DRB1\*1302 and DRB1\*1501 for HLA-DRB1. This set of markers provides unambiguous identification of frequent HLA alleles with 93.4 - 100 % certainty in HLA-A, 97.6 - 100 % in HLA-B, and 97.2 - 100 % in HLA-DRB1.

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A second set of 10 positions each in HLA-A, HLA-B, and HLA-DRB1, respectively are described that provide a maximum number of subgroups, that can then be further resolved by the addition of a set of subgroup specific positions. Again the ten positions in each locus were chosen on the basis of providing best distinction between the frequent HLA alleles listed above from the rest of the HLA alleles (rare). This resulted in groups containing 2-30 HLA alleles depending on the locus. Within each group a number of positions can be tested to provide resolution between the HLA alleles within the group. The number of positions that have to be additionally analysed range from 1-25 in order to achieve 4-digit resolution. With this technology HLA typing can be carried out at a substantially reduced cost with a proven high-throughput detection platform (MALDI mass spectrometry).

In a preferred embodiment of the method of the invention, the DNA strand of step a) is produced by a DNA replication procedure such as PCR or rolling circle replication.

A set of locus-specific PCR reactions for the selective amplification of each locus is described by the International Histocompatibility Working Group, Technical Manuals (Hurly, Fernandes-Vina, Gao, Middleton, Noreen, Ren and Smith; www.ihwg.org/tmanual/Tmcontents.htm).

In a very preferred embodiment of the method of the invention, a combination of primers (pools of primers) contains slightly varying sequences so that all known sequences of the HLA alleles are accommodated by a perfectly matching primer.

The pool of primers guarantees that at least one primer is perfectly matched. The hybridised oligonucleotides of the primer pool are extended onto a polymorphic position. A requirement is that the added base together with the base composition of the primer gives a unique mass. The detection of this mass in the mass spectrometric profile indicates the presence of a sequence containing both the complementary sequence of the primer and the added base. In order to make all

primers of a primer pool distinguishable by mass it is possible to add different mass shifting agents to the primers. The easiest way to accomplish this is by using charge/mass tagging technology such as is used in the GOOD assay. The penultimate base from the 3'end of the primer is amino-modified and used to add tags via NHS-ester chemistry. The pools of primers of course contain primers that sometimes differ by as little as one base. Sequences identical in base content can still be distinguished by the suitable selection of mass tags. Also, we have found that a primer carrying a mismatch in the last eight bases from the 3'end even if it anneals is not extended by the polymerase and thus screened out. This might be due to insufficient hybridisation or a resistance of the DNA polymerase to attach or extend when a mismatch is present. We thus make use of two effects for our minihaplotyping: 1) allele-specific hybridisation and 2) allele-specific primer extension. Mismatches that are further than four bases away from the 3'end of the extension primer do not result in increased complexity of the mass spectra as they are removed in the 5'phosphodiesterase digestion step of the GOOD assay.

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In a preferred embodiment of the method of the invention, mass shifting tags are added to the individual primers sequences of a primer pool to make them uniquely distinguishable once the terminating base is added.

In another preferred embodiment of the method of the invention, termination products for know alleles are generated by extending the perfectly hybridised primer with a combination of dNTPs and ddNTPs or analogues thereof with a DNA polymerase to generate specific termination products to make them uniquely distinguishable by their mass.

In a preferred embodiment of the method of the invention, the GOOD assay is used. It typically applies single base primer extension, thus only the four terminating bases (ddNTPs) or synthetic analogues with the same qualities in terms of DNA polymerase tolerance are used for primer extension.  $\alpha$ -S-ddNTPs are very suitable analogues.

In a preferred embodiment of the method of the invention, mass spectrometry, in particular MALDI or ESI mass spectrometry is used for analysis of the masses of products.

For HLA typing a set of said mini-haplotyping assays has to be carried out to achieve sufficient information content.

For HLA typing of HLA-A the preferred set of assays are those of positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81, 268, 559, 92, 123 and 396 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1; see Figure 1). This results in medium resolution HLA typing. The input criteria for the selection are the frequency of HLA alleles. Some HLA types are identified unambiguously.

For HLA typing of HLA-B accordingly the following positions are preferably analysed by mini-haplotyping assays to achieve medium resolution: 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1; see Figure 2).

For HLA typing of HLA-DRB1 accordingly the following positions are preferably analysed by mini-haplotyping to achieve medium resolution: 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at cDNA sequence position 1 of exon 1; see Figure 3).

In a preferred embodiment for high resolution HLA typing of HLA-A positions 98, 414, 539, 282, 571, 368, 256, 292, 238 and 270 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1; see Figure 4) are used for mini-haplotyping to generate sub-groups (HLA-A\_A, HLA-A\_B, HLA-A\_C, HLA-A\_D, HLA-A\_E, HLA-A\_F, HLA-A\_G, HLA-A\_H, HLA-A\_I, HLA-A\_I, HLA-A\_I, HLA-A\_N, and HLA-A\_O; see Table I).

Positions 224, 268, 376, 502, 561, and 616, are preferably analyzed to generate the graphs.

Positions 224, 268, 376, 502, 561 and 616 are preferably analysed to resolve subgroup HLA-A\_A (sequences identical over exons 2 and 3 for alleles A\*29010101 and A\*29010102); positions 126 and 526 to resolve subgroup HLA-A\_B; positions 81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420, 427, 453, 485, 489 and 502 to resolve subgroup HLA-A\_C (sequences identical over exons 2 and 3 for alleles A\*24020101, A\*24020102L, A\*240203, A\*2409N and A\*2411N); positions 160, 200, 362 and 524 to resolve subgroup HLA-A\_D; positions 180, 299,

positions 160, 200, 362 and 524 to resolve subgroup HLA-A\_D; positions 180, 299, 301, 302, 346, 418, 453, 517, 524, 526, 527, 557, 559 and 560 to resolve subgroup HLA-A\_E; positions 299, 301, 302, 341 and 583 to resolve subgroup HLA-A\_F;



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positions 127, 341, 399, 480, 502, 503, 524, 526, 527, 553, 559, 560 and 565 to resolve subgroup HLA-A\_G; positions 228, 233, 463, 519, 530 and 583 to resolve subgroup HLA-A\_H; positions 102, 275, 317, 362, 418, 419, 497, 524, 555, 595 and 618 to resolve subgroup HLA-A\_I (sequences identical over exons 2 and 3 for alleles A\*680102 and A\*6811N); positions 92, 331, 453, 524, 559, 560 and 564 to resolve subgroup HLA-A\_J; positions 78, 81, 123, 125, 142, 144, 194, 268, 294, 324, 355, 362, 396, 403, 419, 453, 456, 477, 493, 517, 524, 526, 527, 559 and 560 to resolve subgroup HLA-A\_K (sequences identical over exons 2 and 3 for alleles A\*02010101, A\*02010102, A\*020108, A\*0209, A\*0243N and A\*0266); positions 113, 299, 301, 302, 308, 311, 523, 524 to resolve subgroup HLA-A\_L; positions 171, 363, 498 and 559 to resolve subgroup HLA-A\_N; position 299 to resolve subgroup HLA-A\_O.

# TABLE I

Subgroups of	Alleles of Subgroups	Positions to resolve
HLA-A		Subgroups
HLA-A A	A*29010101, A*29010102, A*290201, A*290202,	224, 268, 376, 502, 561,
_	A*2904, A*2906, A*2908N, A*2909	616
HLA-A_B	A*3002, A*3009, A*3012	126, 526
HLA-A C	A*24020101, A*24020102L, A*240202, A*240203,	81, 90, 92, 212, 214, 257,
	A*240204, A*2404, A*2405, A*2408, A*2409N,	265, 299, 302, 404, 420,
	A*2411N, A*2420, A*2421, A*2425, A*2426, A*2427,	427, 453, 485, 485, 489, 502
	A*2429, A*2432, A*2435, A*2436N, A*2437, A*2438,	302
1	A*2439	
HLA-A D	A*0206, A*0214, A*0221, A*0251, A*0257	160, 200, 362, 524
HLA-A E	A*250101, A*250102, A*2601, A*2604, A*2605,	180, 299, 301, 302, 346,
1.1.7.12	A*2609, A*2610, A*2611N, A*2612, A*2614, A*2615,	418, 453, 517, 524, 526,
	A*2617, A*2618, A*6603	527, 557, 559, 560
HLA-A F	A*2502, A*2613, A*6601, A*6602, A*6604	200 201 202 241 592
HLA-A_G	A*110101, A*110102, A*1102, A*1103, A*1104,	299, 301, 302, 341, 583
111111111111111111111111111111111111111	A*1105, A*1107, A*1109, A*1112, A*1113, A*1114,	127, 341, 399, 480, 502, 503, 524, 526, 527, 553,
	A*1115	559, 560, 565
HLA-A_H	A*3301, A*330301, A*330302, A*3304, A*3305,	200 222 462 510 520
1	A*3306, A*3307	228, 233, 463, 519, 530, 583
HLA-A I	A*680101, A*680102, A*680103, A*6807, A*6811N,	102, 275, 317, 362, 418,
	A*6812, A*6816, A*6817, A*6819, A*6821, A*6822,	419, 497, 524, 555, 595,
	A*6823, A*6824	618
HLA-A J	A*2301, A*2303, A*2305, A*2306, A*2307N,	92, 331, 453, 524, 556,
_	A*2308N, A*2310, A*2413	560, 564
HLA-A K	A*02010101, A*02010102, A*020102, A*020103,	78, 81, 123, 125, 142,
—	A*020104, A*020105, A*020106, A*020107,	144, 194, 268, 294, 324,
	A*020108, A*020109, A*0204, A*0209, A*0216,	355, 362, 396, 403, 419, 453, 419, 453, 456, 477,
	A*0224, A*0225, A*0226, A*0229, A*0230, A*0231,	493, 517, 524, 526, 527,
	A*0232N, 0A*0240, A*0242, A*0243N, A*0258,	559, 560
	A*0259, A*0260, A*0264, A*0266, A*0267, A*0253N	
HLA-A_L	A*3201, A*3203, A*3206, A*7401, A*7402, A*7403,	113, 299, 301, 302, 308,
	A*7408, A*7409	311, 523, 524
HLA-A_M	A*010101, A*010102, A*0103, A*0104N, A*0108,	171, 363, 498, 559
	A*0109	
HLA-A_N	A*03010101, A*03010102, A*0303N, A*0304, A*0305,	376, 426, 527, 555, 557,
	A*0306, A*0307, A*0311N	595
HLA-A_O	A*2504, A*2608	299
<u></u>	· · · · · · · · · · · · · · · · · · ·	

In a preferred embodiment for high resolution, HLA typing of HLA-B positions 539, 419, 559, 412, 272, 362, 302, 363, 206 and 369 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1; see Figure 5) are used for mini-haplotyping to generate sub-groups (HLA-B A, HLA-B B, HLA-B C, HLA-B\_D, HLA-B\_E, HLA-B\_F, HLA-B\_G, HLA-B\_H, HLA-B\_I, HLA-B\_J, HLA-B K, HLA-B L, HLA-B M, HLA-B N, HLA-B O, HLA-B P, HLA-B Q, HLA-B\_R, HLA-B\_S, HLA-B\_T, HLA-B\_U, HLA-B\_V, HLA-B\_W, HLA-B\_X, HLA-B Y, HLA-B Z, HLA-B AA, HLA-B AB and HLA-B AC; see Table II). Positions 259, 341 and 473 are preferably analyzed to resolve subgroup HLA-B A (sequences identical over exons 2 and 3 for alleles B\*0801 and B\*0819N); positions 106, 144, 222, 259, 273, 311, 313, 418, 445, 493, 528 and 540 to resolve subgroup HLA-B B (sequences identical over exons 2 and 3 for alleles B\*44020101, B\*44020102, B\*4419N and B\*4427); positions 319, 416, 545 and 572 to resolve subgroup HLA-B C; positions 106, 131, 165, 215, 243, 277, 292, 322, 481, 582, 603 and 616 to resolve subgroup HLA-B D; positions 106, 146, 165, 181, 238, 259, 263, 292, 328.1/329(insert for B\*1579N), 379, 435, 453, 463, 485, 526, 571, 572 and 583 to resolve subgroup HLA-B E (sequences identical over exons 2 and 3 for alleles B\*15010101 and B\*15010102); positions 142, 171, 255, 257, 395, 430, 544, 566 and 572 to resolve subgroup HLA-B F; positions 117, 247, 248, 277, 345, 418, 489 and 527 to resolve subgroup HLA-B G (sequences identical over exons 2 and 3 for alleles B\*270502, B\*270504 and B\*2713); positions 134, 141, 200, 213, 259, 304 and 527 to resolve subgroup HLA-B H; positions 83, 141, 211, 222, 242, 322, 404, 414, 435, 463, 502, 527, 544, 571, 572 and 583 to resolve subgroup HLA-B\_I (sequences identical over exons 2 and for alleles B\*510101, B\*510105, B\*5111N, B\*5130 and B\*5132); positions 103, 142, 222, 243, 259, 292, 477, 486 and 499 to resolve subgroup HLA-B\_J (sequences identical over exons 2 and 3 for alleles B\*400101 and B\*400102); positions 103, 259, 292, 295, 527 and 583 to resolve subgroup HLA-B K (sequences identical over exons 2 and 3 for alleles B\*180101 and B\*1817N); positions 320 and 500 to resolve subgroup HLA-B L; positions 311, 527 and 583 to resolve subgroup HLA-B M; positions 119, 292, 259, 319, 425, 527, 546 and 583 to resolve subgroup HLA-B\_N (sequences identical over exons 2 and 3 for alleles B\*350101, B\*3540N and B\*3542); positions 97, 142, 245 and 527 to resolve subgroup HLA-B\_O; positions 97 and 175 to resolve subgroup HLA-B P; positions

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# TABLE II

[G.1	102.1	
1	Alleles of the subgroup	Positions to resolve
HLA-B		<u>Subgroups</u>
HLA-B_A	B*0801, B*0808N, B*0810, B*0818, B*0819N	259, 341, 473
HLA-B_B	B*44020101, B*44020102S, B*440202, B*440203,	106, 144, 222, 259, 273
· I	B*4405, B*4411, B*4412, B*4419N, B*4422, B*4423N,	311, 313, 418 445, 493,
	B*4424, B*4425, B*4427, B*4433, B*4434, B*4435	528, 540
HLA-B_C	B*4415, B*4501, B*4503, B*4504, B*4505	319, 416, 545, 572
HLA-B_D	B*070201, B*070202, B*070203, B*070204, B*0703,	106, 131, 165, 215, 243,
	B*0716, B*0721, B*0722, B*0723, B*0729, B*0730,	277, 292, 322, 481, 582,
	B*0733, B*0735	603,616
HLA-B_E	B*15010101, B*15010102, B*150102, B*150103,	106, 146, 165, 181, 238,
	B*150104, B*1512, B*1514, B*1515, B*1519, B*1528,	259, 263, 292,
	B*1533, B*1534, B*1538, B*1560, B*1570, B*1571,	328.1/329, 379, 435,
	B*1575, B*1578, B*1579N, B*1581, B*1582	453, 463, 485, 526, 571,
		572,583
HLA-B_F	B*440301, B*4413, B*4426, B*4429, B*4430, B*4432,	142, 171, 255, 257, 395,
	B*4436, B*4437, B*4438, B*4439	430, 544, 566 , 572
HLA-B_G	B*2703, B*270502, B*270503, B*270504, B*270505,	117, 247, 248, 277, 345,
	B*270506, B*2709, B*2710, B*2713, B*2716, B*2717	418, 489 , 527
HLA-B_H	B*5107, B*520101, B*520102, B*520103, B*520104,	134, 141, 200, 213, 259,
	B*5203, B*5204, B*5205	304,527
HLA-B_I	B*510101, B*510102, B*510103, B*510104, B*510105,	83, 141, 211, 222, 242,
	B*510201, B*510202, B*5103, B*5109, B*5111N,	322, 404, 414, 435, 463,
	B*5112, B*5114, B*5118, B*5119, B*5123, B*5124,	502, 527, 544, 571, 572,
	B*5126, B*5127N, B*5128, B*5130, B*5132, B*5133	583
HLA-B_J	B*400101, B*400102, B*400103, B*4010, B*4011,	103, 142, 222, 243, 259,
	B*401401, B*401402, B*401403, B*4022N, B*4025,	292, 477, 486 , 499
	B*4043	
HLA-B_K	B*180101, B*180102, B*1803, B*1804, B*1805,	103, 259, 292, 295, 527,
	B*1811, B*1812, B*1815, B*1817N	583
HLA-B_L	B*570101, B*5706, B*5708	320, 500
HLA-B_M	B*3527, B*5301, B*5302, B*5306, B*5308	311, 527, 583
HLA-B_N	B*350101, B*350102, B*3507, B*3510, B*3511,	
	B*3521, B*3524, B*3529, B*3540N, B*3541, B*3542,	
	B*5305	. ,
HLA-B_O	B*5501, B*5502, B*5505, B*5510, B*5516	97, 142, 245, 527
	B*5401, B*5402, B*5507	97, 175
	*	





HLA-B_Q	B*3910, B*670101, B*670102	246, 277
HLA-B_R	B*3803, B*390201, B*390202, B*3913, B*3923	246, 292, 311, 503
HLA-B_S	B*3801, B*380201, B*380202, B*3804, B*3805, B*3809	103, 261, 309, 311, 474
HLA-B_T	B*390101, B*390103, B*390104, B*3904, B*3905,	97, 103, 106, 243, 259,
	B*3912, B*3922, B*3925N, B*3926	292, 404 , 524
HLA-B_U	B*3503, B*3513, B*3536	259,320
HLA-B_V	B*0734, B*5504	106
HLA-B_W	B*4047, B* 4431	97
HLA-B_X	B*4002, B*4027, B*4029, B*4035, B*4040, B*4045	97, 106, 257, 418, 463
HLA-B_Y	B*400104, B*4004	106
HLA-B_Z	B*4012, B*4046, B*4803	106, 144
HLA-B_AA	B*2703, B*270502, B*270503, B*270504, B*270505,	117, 247, 248, 283, 345,
	B*270506, B*2709, B*2710, B*2713, B*2716, B*2717	418, 489, 527
HLA-B_AB	B*1562, B*4802	106
HLA-B_AC	B*1302, B*1308	548

246 and 277 to resolve subgroup HLA-B\_Q; positions 246, 292, 311 and 503 to resolve subgroup HLA-B\_R; positions 103, 261, 309, 311 and 474 to resolve subgroup HLA-B\_S; positions 97, 103, 106, 243, 259, 292, 404 and 524 to resolve subgroup HLA-B\_T (sequences identical over exons 2 and 3 for alleleles B\*390101 and B\*390103); positions 259 and 320 to resolve subgroup HLA-B\_U; position 106 to resolve HLA-B\_V; positions 97 to resolve HLA-B\_W; positions 97, 106, 257, 418 and 463 to resolve HLA-B\_X; position 106 to resolve HLA-B\_Y; positions 106 and 144 to resolve HLA-B\_Z; positions 117, 247, 248, 283, 345, 418, 489, and 527 to resolve HLA-B\_AA; positions 106 to resolve HLA-B\_AB; positions 548 to resolve HLA-B\_AA.

In a preferred embodiment, the method for HLA typing resolves groups A-P of HLA-DRB1.

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For high resolution, HLA typing of HLA-DRB1 positions are: 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at DNA sequence position 1 of exon 1; see Figure 6) are used for minihaplotyping to generate sub-groups (HLA-DRB1\_A, HLA-DRB1\_B, HLA-DRB1\_C, HLA-DRB1\_D, HLA-DRB1\_E, HLA-DRB1\_F, HLA-DRB1\_G, HLA-DRB1\_H, HLA-DRB1\_I, HLA-DRB1\_J, HLA-DRB1\_K, HLA-DRB1\_L, HLA-DRB1\_M, HLA-DRB1\_N, HLA-DRB1\_O, HLA-DRB1\_P; see Table III).

In a very preferred embodiment, positions 123, 174, 250, 278 and 317 are analysed to resolve subgroup HLA-DRB1 A; positions 192, 203, 256 and 259 to resolve subgroup HLA-DRB1 B; 256, 260, 317 and 351 to resolve subgroup HLA-DRB1 C; positions 155, 204, 233, 239, 256, 304, 357 and 366 to resolve subgroup HLA-DRB1\_D; positions 122, 171, 257 and 317 to resolve subgroup HLA-DRB1 E; positions 164, 167, 171, 230, 235, 306, 317, 321 and 337 to resolve subgroup HLA-DRB1\_F; positions 164, 257, 266 and 303 to resolve subgroup HLA-DRB1\_G; positions 164, 181, 188, 220, 229, 256, 266, 317 and 318 to resolve subgroup HLA-DRB1\_H; position 257 to resolve subgroup HLA-DRB1 I; positions 181, 239 and 357 to resolve subgroup HLA-DRB1\_J; positions 122, 144, 239, 303, 317, 318 and 321 to resolve subgroup HLA-DRB1 K (sequences identical over exons 2 and 3 for alleles DRB1\*110101 and DRB1\*110102); positions 118, 161, 257, 260, 318 and 321 to resolve subgroup HLA-DRB1 L; positions 165, 257, 293 and 303 to resolve subgroup HLA-DRB1 M (sequences identical over exons 2 and 3 for alleles DRB1\*120101 and DRB1\*1206); positions 177, 240, 256, 257 and 357 to resolve subgroup HLA-DRB1\_N; positions 150 175, 230, 236 and 321 to resolve subgroup HLA-DRB1\_O (sequences identical over exons 2 and 3 for alleles DRB1\*150101 and DRB1\*1513); positions 115, 220 and 317 to resolve subgroup HLA-DRB1\_P.

Another object of the invention is a kit to carry out the procedure. It consists of pooled combinations of primers. The primers that are used in the pools for HLA-A, HLA-B, and HLA-DRB1 and the masses of the genotyping products are listed in Tables IV, V, and VI respectively. CT refers to the mass shifting mass tag that is attached to that primer of the pool.

Another object of the invention is the use of the method of the invention for screening of tissue donors.

In a preferred embodiment, the use is for bone marrow donors in registries for screening of frequent and rare HLA types.

Still another object of the invention is the use of the primers represented in Table IV, V and VI to carry out HLA typing.

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### TABLE III

Subgroups of	Alieles of Subgroups	Positions to resolve
HLA-DRB1	Tanolog or buogroups	Subgroups
HLA-	DRB1*070101, DRB1*070102, DRB1*0703, DRB1*0704,	123 174 250 317
DRB1_A	DRB1*0705, DRB1*0707	123, 174, 230, 317
HLA-	DRB1*040101, DRB1*040102, DRB1*0409, DRB1*0426,	192 203 256 259
DRB1 B	DRB1*0433	1,200,200,200
HLA-	DRB1*0404, DRB1*0410, DRB1*0423, DRB1*0440,	256 260 317 351
DRB1_C	DRB1*0444	250, 200, 517, 551
HLA-	DRB1*040501, DRB1*040502, DRB1*040503,	155, 204, 233, 239,
DRB1_D	DRB1*040504, DRB1*0408, DRB1*0429, DRB1*0430,	256, 304, 357, 366
	DRB1*0445, DRB1*0448	
HLA-	DRB1*1402, DRB1*1409, DRB1*1413, DRB1*1446,	122, 171, 257, 317
DRB1_E	DRB1*1447, DRB1*1448	·
HLA-	DRB1*130101, DRB1*130102, DRB1*130103,	164, 167, 171, 230,
DRB1_F	DRB1*1315, DRB1* 1327,	235, 306, 317, 321,
		337
HLA-	DRB1*130201, DRB1*130202, DRB1*1331, DRB1*1339,	164, 257, 266, 303
DRB1_G	DRB1*1341	
HLA-	DRB1*030101, DRB1*030102, DRB1*0307, DRB1*0312,	164, 181, 188, 220,
DRB1_H	DRB1*0313, DRB1*0315, DRB1*0316, DRB1*0318,	229, 256, 266, 317,
	DRB1*0322, DRB1*0323	318
HLA-	DRB1*1137, DRB1*1425	257
DRB1_I		
HLA-	DRB1*110401, DRB1*110402, DRB1*1143, DRB1*1146	181, 239, 357
DRB1_J		
HLA-	DRB1*110101, DRB1*110102, DRB1*110103,	122, 144, 239, 303,
DRB1_K	DRB1*110104, DRB1*110105, DRB1*112701,	317, 318, 321
	DRB1*112702, DRB1*1130, DRB1*1139	
HLA-	DRB1*1117, DRB1*140101, DRB1*140102, DRB1*1408,	118, 161, 257, 260,
DRB1_L	DRB1*1426, DRB1*1438, DRB1*1439	318, 321
HLA-	DRB1*120101, DRB1*120102, DRB1*1206, DRB1*1207,	165, 257, 293, 303
DRB1_M	DRB1*1208, DRB1*1209	-
HLA-	DRB1*080101, DRB1*080102, DRB1*080201,	177, 240, 256, 257,
DRB1_N	DRB1*080202, DRB1*080203, DRB1*0807, DRB1*0811	357
HLA-	DRB1*150101, DRB1*150103, DRB1*150105,	150 175, 230, 236,
DRB1_O	DRB1*1503, DRB1*1506, DRB1*1509, DRB1*1513	321
HLA-	DRB1*010101, DRB1*0105, DRB1*0107, DRB1*0111	115, 220, 317
DRB1_P		
<del></del>	<u> </u>	l





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		TABLE IV						
No	Name	Saguence	CT.	Primer Masses	Α	С	G	T
1		Sequence TGCTCGCCCCAGGCTCCspC^spA	To	1098,1	14405 4	14404.0		7
1 2		TGCTCGCCCCAGGCTCCspC spA	1 6	1113,1	1425,1	1401,3 1416,3		<del> </del> -
		, , , , , , , , , , , , , , , , , , ,	<del>Ť</del>	1110,1	<del></del>	1410,3	1452,4	-
3	HLAA_921_1f20	AGGCTCCCACTCCATGAGspC^spT	0	1129,1	1456,4	<del> </del>		<u> </u>
4		AGGCTCCCAMTCCATGAGspG^spT	0	1169,1	1496,4		1512,4	
5	HLAA_923_1f20	AGGCTCTCASTCCATGAGspG^spT	0	1169,1	1496,4		1512,4	
								<b> </b>
5 6		CCACTCCATGAGGTATTTspC^spA	0	1113,1	-	1416,3	-	
7	HLAA_982_1f20	CCACTCCATGAGGTATTTspC^spT	0	1104,1	1431,4	1407,3	~	1422,3
-	UI AA 4224 2=20	CCCATCAACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	<u> </u>					
ြိ	HI AA 4222 2:20	GCGATGAAGCGGGGCTCspCspT^spC GCGATGAAGCGGGGCTCspTspC^spC	0			<u> </u>	1853,8	
	HI AA 1232 2120	GCGATGAAGCGGGGCTCspTspC-spC GCGATGAAGCGGGGCTTspCspC-spC	-28		1707,7	-		
111	HI AA 1234 2020	GMGATGAAGCGGGGCTTspCspC*spC GMGATGAAGCGGGGCTCspCspC	0	1408,4	4700 7	<u> </u>	1751,6	
- <del></del>	11204 2120	OMONTONOCOGOGO TOSPOSPO-SPC	١٠	1393,4	1720,7		1736,7	
12	HLAA 2381 2r20	CTSGTCCCAATACTCCGspGspA^spC	0	1497,4		1800,6		
1 13	HLAA 2382 2r20	CYCGTCCCAATACTCCGspGspA^spC	ŏ	1497,4		1800,6		<u> </u>
0 14	HLAA 2383 2r20	CTCGTCCCAATACTCCGspGspC^spT	ō	1488,4	<del>-</del>	1791,6		1806,4
15	HLAA 2384 2r20	CTSGTCCCAATACTCAGspGspC^spC	ō	1473,4	-	1776,6	-	1000,4
16	HLAA_2385_2r20	CYGGTCCCAATACTCCGspGspC^spC	0	1473,4		1776,6		<del></del>
17	HLAA_2386_2r20	CMGGTCCCAATACTCCGspGspC^spC	0	1473,4	•	1776,6		
18	HLAA_2387_2r20	CYCGTCCCAATACTCCGspGspC^spC	0	1473,4	-	1776,6	-	-
<u> </u>								
19	HLAA_2561_1r19	CTTCATATTCCGTGTCTCspC^spT	0	1089,1	•	1392,3	1432,4	-
20	HLAA 2562 1r19	CTTCACWTTCCGTGTCTCspC^spT	0	1089,1	*	1392,3	1432,4	
$5 \frac{21}{22}$	HLAA 2563 1719	CTTCACATKCCGTGTCTGspC^spA	0	1138,1	-	-	1481,4	
	HI AA 2565 1-10	CTTCACTTTCCGTGTGTTspC^spC CYTCACATTCCGTGTGTTspC^spC	0	1089,1	-		1432,1	•
24	HI AA 2566 1r19	CTTCACATTCCGTGTGTTspC-spC CTTCACRTTCCGTGTCTCspC-spC	00	1089,1	-	4077.0	1432,1	-
25	HLAA 2567 1r19	CTTCASTTGCCGTGTCTCspC-spC	ö	1074,1 1074,1		1377,3	1417,4	
26	HLAA 2568 1r19	CTTCAGTTKCCGTGTCTCspC^spC	ö	1074,1	-	1377,3 1377,3		-
			Ť	1074,7		1377,3	1417,4	
28	HLAA_2681_1f20	ATTGGGACCGGAACACACspG^spG	0	1154,1	1481.4	1457,3		
29	HLAA_2682_1f20	ATTGGGACCTGCAGACACspG^spG	0	1154,1	1481,4	1457,3	-	-
30	HLAA 2683 1f20	ATTGGGACSAGGAGACACspG^spG	0	1154,1	1481,4	1457,3	_	-
0 31	HLAA_2684_1f20	ATTGGGACSGGGAGACACspG^spG	0	1154,1	1481,4	1457,3		-
32	HLAA_2685_1f20	ATTGGGACSAGGAGACAGspG^spG	0	1194,1	1521,4	•		-
- 22								
33	HLAA 2701 1719	CTGTGAGTGGGCCTTCspA^spT	0	1113,1	1440,4	-	-	-
35	HI AA 2702 4-40	CTGTGACTGGGCCYTCspA^spC CTGTGAGTGGSCCTTCspA^spC	-14	1084,1	1411,4		1427,4	1402,4
100	11LAN 2103 1119	CTGTGAGTGGSCCTTCSpA^spC	-14	1084,1	1411,4		1427,4	1402,4
36	HLAA 2821 1520	ACACGGAATGTGARGGGCspC^spA		4000 4		4404.0	4444.5	
37	HLAA 2822 1f20	ACASGGAAAGTGAAGGCCspC^spA	0	1098,1 1098,1		1401,3 1401,3		-
20	HLAA 2823 1f20	ACACGGCAWGTGAAGGCCspC^spA	8	1098,1		1401,3		-
$\frac{30}{39}$	HLAA 2824 1f20	ACACGGAACGTGAAGGCCspC^spA	ŏ	1098,1		1401,3		
40	HLAA 2825 1f20	ACACGGAATRTGAAGGCCspC^spA	ŏ	1098,1		1401,3		
			H			1401,3	1441,3	
41	HLAA_2921_2f20	TGAAGGCCCACTCACAGspAspG^spT	-14	1498,4		1801,6		
42	HLAA_2922_2f20	TGAAGGCCCACTCACAGspGspC^spT	0	1488,4			1831,7	
43	HLAA_2923_2f20	TGAAGGSCCACTCACAGspAspT^spT	0	1589,6	-		1932,9	-
44	HLAA_2924_2f20	TGARGGCCCAGTCACAGspAspC^spT	0	1427,4	-	1775,6		-
45	HLAA_2925_2f20	TGAAGGCCCASTCACAGspAspC^spT	0	1427,4		1775,6		_
` <b> </b>								
70	IHLAA 3681 1f20	TCACACCATCCAGATAATspG^spC	0		1456,4			-
4/	HLAA 3682 1f20	TCACACCATCCAGMTAATspG^spT	0			1447,1		
48	ITLAA 3683 1f20	TCACACCSTCCAGAGGATspG^spT	0	1144,1	1471,6	1447,1	1487,4	1462,3
49	TLAA_3684_1120	TCACACCVTCCAGATGATspG^spT	0	1144,1	1471,6	1447,1	1487,4	1462,3
50	HI AA 3061 3-20	CCTCCTACCCCCCCAC						
		GCTGGTACCCGCGGAGspGspA^spG	0	1537,4	1		1880,7	

		,,,,,,		<b>,</b>					
			GCCGGTACCCGCGGAGspTspA^spA	0	1496,4	•		1839,7	-
			GGTGGTACCCGYGCAGspGspA^spA	0	1496,4		•	1839,7	
			GGTGGTACCCGCAGAGspGspA^spA	0	1521,5	•	-	1864,8	
			GTTCATACCCGCGGAGspGspA^spA	0	1521,5	•	-	1864,8	
			GSTGGTACCCGCGGAGspGspA^spA	0	1521,5	-	-	1864,8	
	56	HLAA_3967_2r20	GCCGGTACCCGCGGAGspGspA^spA	0	1521,5	-	•	1864,8	1839,
	57	HLAA_4141_1f20	CGCTTCCTCCGCGGGTATspG^spA	0	1153,1	1480,1		•	-
			CGCTTCCTCTGCGGGTACspC^spA	0	1098,1	•		1441,4	-
;			CGCTTCCTGCGCGGGTACspC^spA	0	1098,1	-		1441,4	-
			CGCTTCCTCCACGGGTACspC^spA	0	1098,1	-		1441,4	
			CGMTTCCTCCGCGGGTACspC^spA	0	1098,1	-		1441,4	-
			CGCCTCCTCCGCGGGTACspC^spA	0	1098,1			1441,4	
			CACTTCCTCCGCGGGTACspC^spG	ō	1114,1	-		1457,4	
	64	HLAA_4148_1f20	CGCTTMCTCCGCGGGTACspC^spG	0	1114,1	-		1457,4	
	0.7	111 AA 4804 4 55	OTCOM ON COOK OF THE CO	ابرا	4000				
			GTCCAAGAGCGCAGGTCTspT^spC	0	1206,2	-	-	45645	1524,
			GTCCAGAGCGCAGGTCCspT^spC	0	1191,2		-	1534,5	
)	6/	HLAA_4533_1120	GTCCAGGAGCTCAGGTCCspT^spC	0	1191,2			1534,5	1509,
	68	HI AA 5021 2r20	GGCCGYCTCCCACTTGTspGspC^spT	0	1463,4				1781,
			GGCYGCCTCCCACTTGCspGspC^spT	ŏ	1448,4		1751 G	1791,7	
			CGGAGTCTCCCACTTGCspGspC^spT	ö	1448,4	<u> </u>		1791,7	
			GGCCGCCTCCCACTTGCspGspC^spC	-14		_		-	1737,
					, , , , , , , , , , , , , , , , , , ,			<b></b>	· · · · · · · ·
			AGTGGGAGACTCCGCCCAspT^spG	0	1255,3	1582,6	1558,5	_	1573,
			CAAGTGGGAGGCGGYCCAspT^spG	0	1255,3	1582,6	1558,5		1573,
<u>.</u> .	74	HLAA_5273_1f20	CAAGTGGGAGRCGGCCCAspT^spG	0	1255,3	1582,6	1558,5	-	1573,
5	75	HLAA_5274_1f20	CAAGTGGGAGGCGGCCCTspT^spG	0	1246,3	-	-	-	1564,
÷	76	HLAA 5275_1f20	CAAGTGGGAGGCGGCCCGspT^spT	0	1246,3	-	-	1589,6	_
:			CAAGTGGGAGGCGGCCCGspT^spC	0	1231,3	-	-	1574,5	
•			CAAGTGGGAGGCGGCCMGspT^spG	0	1271,3	1598,6		-	1589,
	79	HLAA_5278_1f20	CAAGTGGGAGGCRGCCCGspT^spG	0	1271,3	1598,6	-	-	1589,
	80	HI AA 5304 4640	GCCCRTGAGGCGGAGCAspG^spC	0	1420 4	1465 4		4494 4	4450
			GYCCATGCGGCGGAGCAspG*spC	믕	1138,1 1138,1	1465,4 1465,4	-	1481,4 1481,4	
			GCCGTCGGGCGAGCAspG*spC	6	1138,1	1465,4	-	1481,4	
0			GCCCATGGGCGGAGCASpG*SpC	8	1138,1	1465,4	-	1481,4 1481,4	
-			GTCCATGCGGCGGAGCAspG^spT	ö	1153,1	1400,4	-	1496,4	1471
			GCCGTYGGGCGAGCAspG^spT	6	1153,1			1496,4	
			GCCCATGAGGCGGAGCAspG^spT	ŏ	1153,1	-	-	1496,4	
			GCCCWTGTGGCGGAGCAspG^spT	ō	1153,1	-	-	1496,4	
			GCCMGTGTGGCGGAGCAspG^spT	Ŏ	1153,1	_	_	1496,4	
								•	
			GCGGAGCCACTCCACGCAspC^spT	0	1113,1	-	1416,3	_	
			GCGGAGCCCGTCCACGCAspC^spT	0	1113,1	-	1416,3	•	•
5			GCGGAGCCACTCCACGCAspC^spA	0	1122,1	-	•	1465,4	-
	92	HLAA_5594_1r20	GCGGAGCCCGTCCACTCAspC^spG	0	1138,1	-	•	-	1456,
			GCGGAGCCAGTCCACGCAspC^spG	0	1138,1	-	•	-	1456,
			GCGGAGCCMGTCCACGCAspC^spG	0	1138,1	•	-	-	1456,
			GCGGAGCCACTCCACGCAspC^spC	0	1098,1	1425,4		1441,4	-
	90	HI AA 5500 4.65	GCGGAGCCGTCCACGCAspC^spC	0	1098,1	1425,4	-	1441,4	4400
		1120 PRICE TO STATE	GCGGAGCCACTCCACGCAspG^spG	0	1178,1	-			1496,
	97	HLAA 5711 2520	TGGAGGGCCKGTGCGTGspGspA^spG	0	1537,4	-	-		1855,
_	98	HLAA 5712 2f20	TGGAGGGYGAGTGCGTGspGspA*spG	Ö	1537,4	-			1855,
0	99	HLAA 5713 2f20	TGSAGGGCCGGTGCGTGspGspA^spG	Ö	1537,4	-			1855,
	100	HLAA 5714 2f20	TGGATGSCACGTGCGTGspGspA^spG	Ö	1537,4		-		1855,
	101	HLAA_5715_2f20	TGGAGGGCACSTGCGTGspGspA^spG	Ö	1537,4	-			1855,
	102	HLAA_5716_2f20	TGGAGGGCACGTGMGTGspGspA^spC	ō	1497,4	-	-	1840,7	1815,
	103	HLAA_5717_2f20	TGGAGGCYGGTGCGTGspGspA^spC	0	1497,4	-	-	1840,7	1815,

## TABLE V

Name	<b></b>			<del></del>	Primer				-
1   H.A.B. 971, 2120   CCCACTCCATGAGGCATSTSTST">   1   1403   1843, 1853, 18	No	Name	Sequence	CT	1	Α	C	G	т
H.A.B. 972, 2/20   CCACTYCATGAGGTATEPTSPT*SPC   0   1540,3   1643,7   1883,8   1885,8   1885,4   114.B. 2061,1120   CGACGCCGGAGTCCGMGAGSPC*SpA   28   1150,1   1477,4   1453,3   1468,4   114.B. 2062,1720   CGACGCCGGAGTCCGAGSPC*SpA   29   1150,1   1477,4   1453,3   1468,5   1488,4   114.B. 2063,1720   CGACGCCGGGAGTCCGAGSPA*SPG   0   1178,1   1905,4   1521,4   147,4   1453,3   1468,6   1488,6   14	1	HLAB_971_2f20	CCCACTCCATGAGGCATspTspT^spC	0	1540.3	_			
3	2	HLAB_972_2f20	CCCACTYCATGAGGTATspTspT^spC	0		-			1858.
H.H.B. 2003, 1/120   GAGGCCAGGAGTCCGAGSPG*SpA   28   1150,1   1477,4   1453,3   - 1465,5   1418,0084,1120   GAGGCCGGAGGTCCGAGSPA*SpG   0   1478,1   1505,4   1521,4   - 1465,6   1418,2084,1120   GAGGCCGCGAGGTCCGAGSPA*SpG   0   1478,1   1505,4   1521,4   - 1441,4	-	UI AD 2004 4620	CCACCCCCACTOMOAG						
6 HAB. 2063 1/120 CGACGCCGGAGTCCRAGSpA*spG         0 1175,1 1505,4         1521,4           7 HAB. 2221 1/19 GCCCCTCCTGCTCCACCSpC*spA         0 1098,3 1425,6         1521,4           7 HAB. 2221 1/19 GCCCTCCTGCTCCACCSpC*spA         0 1098,3 1425,6         1441,4           8 HAB. 2221 1/19 GCCCTCCTGCTCACCSpC*spA         0 1098,3 1425,6         1441,4           9 HAB. 2591 2/20 GGCCGGAGTTTGGGACSpGapA*spA         0 1098,3 1425,6         1441,4           10 HAB. 2592 1/20 GGCCGGAGTTTGGGACSpGapA*spG         0 163,3         1425,4         1441,4           11 HAB. 2593 2/20 GGCCGGAGTTTGGGACSpGapA*spG         0 1497,4         1840,7         1840,7           11 HAB. 2593 2/20 GGCCGGAGTTTGGGACSpCSpC*spG         28 1405,4         1772,7         1788,7           12 HAB. 2595 2/20 GGCCGGAGTTTGGGACSpCSpC*spG         28 1445,4         1772,7         1788,7           15 HAB. 2595 2/20 GGCCGGAGATTGGGACSpCSpC*spG         28 1445,4         1772,7         1788,7           16 HAB. 2599 2/20 GGCCGGAGATTGGGACSpCSpC*spG         28 1445,4         1772,7         1788,7           17 HAB. 2599 2/20 GGCCGGAGATTGGGACSpCSpC*spG         28 1445,4         1772,7         1788,7           18 HAB. 2721 1/20 GGACCGGAGACACGGAASpC*spA         0 1122,1         -         -         1788,7           19 HAB. 2772 1/20 GGACCGGAGACACGGAASpC*spACSpC*spAC         0 1122,1         <	-	HLAB_2061_1120	CGACGCCACGAGTCCGAGGGGAGGAGA						
6 H.AB, 2084, 1120 CGACGCORCGAGITCCGAGSpA*spG 0 1478,1 1505,4 . 1521,4		HI AB 2063 1f20	CGACGCCGCGAGTCCRAGenAvenG						1468,
7 HLAB 2221 1/19 GCCCCTCTGCTCCACCSpC*SpA 0 1098,3 1425,4 1441,4		HLAB 2064 1f20	CGACGCCRCGAGTCCGAGspA^spG						<del></del>
8 HLAB 2222, 119 GCCCGGACTATTGGGACSpGepG*spG				<u> </u>		1000,4	— <u> </u>	1021,4	<del>  -</del> -
8 HLAB 2222, 119 GCCCCGGAGTATTGGGACSpGspG*pG 0 1513,4			GCCCCTCCTGCTCCACCspC^spA	0	1098,3	1425,4	-	1441,4	
10   HLAB_2592_2210   GGCCGGAGTATTGGGACSPGSPAPGG	8	HLAB_2222_1r19	GCCCCTCYTGCTCTATCspC^spA	0	1098,3	1425,4	-		-
10   HLAB_2592_2210   GGCCGGAGTATTGGGACSPGSPAPGG	-	HI AR 2501 2620	GCCCGAGTATTCGGACanCanChanG	_	4540.4	<u> </u>			
11   H.A.B. 2593_2220   GGCCGGAGTATTGGGACSPCSpC*pG		HI AB 2592 2f20	GCCGGAGTATTGGGACspGspG-spG						
12   HIAB_2594_2720   GGCCGGAGTATTGGGACSPCSpC*spG   0   1488,4   1772,7   1788,7		HLAB 2593 2120	GGCCGGAGTATTGGGACenCenChenG						
13   HIAB_2595_2720   GGCCGGAGCTTTGGGACSpCspC*spG   28   1445,4   1772,7   1778,7   1788,7		HLAB 2594 2f20	GGCCGGAGTATTGGGATsnCsnG^snG						
HIAB 2595 2220 GGCCGGGAGCATTGGGACSDCSpC*SpC		HLAB 2595 2f20	GGCCGGAGTTTTGGGACsnCsnG^snG						
HIAB 2897 2720   GGCCGGGATATTGGGACSpCspCspCspC -28   1445,4   1772,7   1788,7   17			GGCCGGAGCATTGGGACspCspG^spG						
HIAB 2598 2720   GGCCGGAATATTGGGACSpCspG-spG   28   4445,4   1772,7   1788,7   178		HLAB 2597 2f20	GGCCGGGATATTGGGACspCspG^spG						
The Hab		HLAB 2598 2f20	GGCCRGAATATTGGGACspCspG^spG						
HIAB 28910_2120   GGCCTTAGTATTGGGACSpCspC*spG			GGCGGGMGTATTGGGACspCspG^spG					1788.7	
HIAB 2721 1120   GGACSGGAGACACGGAAspC*spA	18	HLAB 25910 2f20	GGCCTTAGTATTGGGACspCspG^spG			1772.7			
HIAB 2722   1120   GGACGGGACACACGGAASpC^spA								1700,7	<del></del>
HIAB 2723   1120   GGACGGGAACACACAGGASpC*spA		HLAB_2721_1f20	GGACSGGGAGACACGGAAspC^spA	0	1122,1	-	-	-	1440.
HIAB 2772   1120   GGACCGGAACACACAGAAspC*spT   0   1113,1   -   1456,4   -   1393;   23   HIAB 2725   1120   GGACCGGGAACACACAGACSpC*spT   0   1153,1   1480,4   -   -   1393;   24   HIAB 2725   1120   GGACCGGGAGACACAGAASpC*spT   0   1104,1   1431,4   1407,3   1447,4   1422;   25   HIAB 2727   1120   GGACCGGGASACACAGATSpC*spT   0   1104,1   1431,4   1407,3   1447,4   1422;   26   HIAB 2728   1120   GGACCGGGASACACAGATSpC*spT   0   1104,1   1431,4   1407,3   1447,4   1422;   27   HIAB 2728   1120   GGACCGGGACACACAGATSpC*spT   0   1104,1   1431,4   1407,3   1447,4   1422;   27   HIAB 2728   1120   GGACCGGGACACACAGATSpC*spT   0   1104,1   1431,4   1407,3   1447,4   1422;   28   HIAB 2921   2119   CAAGACCACACAGATSpC*spT   0   1458,3   -   1801,6   -     1801,6   -		HLAB_2722_1f20	GGACGRGGAGACACGGAAspC^spA	0		-		-	1440.
HIAB   2725   1720   GGACCGGGAGCACAGAASpG^SpT   0   1153,1   1480,4				0	1113,1	-	•	1456,4	-
A			GGACCGGAACACACAGACspC^spT		1075,1		-	-	1393,
HIAB 2721 1120   GGACCGGGASACACAGATSpC'spT			GGACCGGGAGACACAGAAspG^spT					-	-
26   HLAB 2728   1120   GGACCGGGACACACACACACASTSPC*SPT   0   1104,1   1431,4   1407,3   1447,4   1422,2   1418,2729   1120   GGACCSGGAGACACACACACACSTSPC*SPT   0   1104,1   1431,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1407,3   1447,4   1422,3   1418,4   1414,3   1407,3   1447,4   1422,3   1418,4   1414,3   1407,3   1447,4   1422,3   1418,4   1414,3   1407,3   1447,4   1422,3   1418,4   1414,3   1407,3   1447,4   1423,3   1418,4   1414,3   1407,3   1447,4   1422,3   1414,3   1407,3   1414,3   1407,5   1732,5   1414,3   1407,5   1732,5   1414,3   1407,5   1732,5   1414,3   1414,3   1407,5   1732,5   1414,3		HLAB_2726_1f20	GGACCGGGAGATACAGATspC^spT					1447,4	1422,3
HIAB 2729 1f20 GGACCSGGAGACACAGATspC^spT		HLAB_2727_1f20	GGACCGGGASACACAGATspC^spT				1407,3		1422,3
28 HIAB 2921 2ff9 CAAGACCAACACACAGSpGspC^spT		HLAB 2728 1120	GGACCGGGACACACAGATspC^spT						1422,3
HLAB 2922 2ff9   CAAGSCCCAGGCACAGSPGSPC*SPT   0   1458,3   -   1801,6   -   1757,6   1732,6	21	HLAB_2/29_1120	GGACCSGGAGACACAGA1spC^sp1	0	1104,1	1431,4	1407,3	1447,4	1422,3
HLAB   2922   2ff   CAAGSCCCAGGCACAGSPGSPC*SPT   0   1458,3   -   1801,6   -     30   HLAB   2923   2ff   CAAGACCACAGGSPASPC*SPT   -28   1414,3   -   1757,6   1732	28	HLAB 2921 2f19	CAAGACCAACACACAGenGenC^enT		1/50 2			4004.6	
HLAB 2923 2ff9   CAAGACCACACACACGGSPASPC^SpT   -28		HLAB 2922 2f19	CAAGSCCCAGGCACAGSpGspC^spT						
HLAB 2924 2ft9   GAAGGCCTCCGCGCAGSpAspC^spT   -28   1414,3   -   1757,6   1732,5	30	HLAB 2923 2f19	CAAGACCAACACACGGspAspC^spT						17225
HLAB 2925 2f19   CAAGGCCMAGGCACAGSPASPC^SPT   -28   1414,3   -   1757,6   1732,5	31	HLAB 2924 2f19	GAAGGCCTCCGCGCAGspAspC^spT						4732,
33   HLAB 2926 2ff9   CAAGSGCCAGGCACAGSPASPC^spT   -28   1414,3   -     -   1757,6   1732,5	32		CAAGGCCMAGGCACAGspAspC^spT						1732.5
HLAB 2927 2f19   GAAGACCAACACACAGSPASPC^SpT   -28   1414,3   -   1757,6   1732,5	33	HLAB_2926_2f19	CAAGSGCCAGGCACAGspAspC^spT			-			
Start   Star	34	HLAB_2927_2f19	GAAGACCAACACACAGspAspC^spT	-28				1757,6	
HLAB 30211 2ft9   ACACAGACTTACAGAGSPASPG^SPA   -28   1493,5   1820,8   -   1836,8   -   -   -   -     -									
37	35	HLAB_3021_2f19	GCACAGACTGACCGAGspTspG^spG		1528,4		•	1871,7	-
38         HLAB         3023         2f19         RCACAGACTGACCGAGSpAspG^spG         0         1537,4         1864,7         -         -         -           39         HLAB         3024         2f19         GCACAGACTGCCGAGSpTspG^spA         -28         1481,4         1811,7         -         1827,7         -           40         HLAB         3025         2f19         ACACAGACTGACCGAGSpTspG^spA         -28         1481,4         1811,7         -         1827,7         -           41         HLAB         3026         2f19         RCACAGACTGACCGAGSpASpG^spA         -28         1493,5         1820,8         -         1827,7         -           42         HLAB         3027         2f19         ACACAGACTGACCGAGSpASpG^spA         -28         1493,5         1820,8         -         1836,8         -           43         HLAB         3029         2f19         GCRCAGACTTACCGAGSpASpG^spA         -28         1493,5         1820,8         -         1836,8         -           45         HLAB         30210         2f19         ACACRGACTTACCGAGSpASpG^spA         -28         1493,5         1820,8         -         1836,8         -           45         HLAB         3621         2f20 </td <td>36</td> <td>HLAB_30211_2f19</td> <td>ACACAGACTTACAGAGspAspG^spA</td> <td></td> <td>1493,5</td> <td></td> <td>•</td> <td>1836,8</td> <td>-</td>	36	HLAB_30211_2f19	ACACAGACTTACAGAGspAspG^spA		1493,5		•	1836,8	-
39 HLAB 3024 2ff9 GCACAGACTGGCCGAGSpTspG^spA -28 1481,4 1811,7 - 1827,7 - 40 HLAB 3025 2ff9 ACACAGACTTACCGAGSpTspG^spA -28 1481,4 1811,7 - 1827,7 - 41 HLAB 3026 2ff9 RCACAGACTGACCGAGSpTspG^spA -28 1481,4 1811,7 - 1827,7 - 42 HLAB 3027 2ff9 ACACAGGCTGACCGAGSpASpG^spA -28 1493,5 1820,8 - 1836,8 - 43 HLAB 3028 2ff9 RCACAGACTGACCGAGSpASpG^spA -28 1493,5 1820,8 - 1836,8 - 44 HLAB 3029 2ff9 GCRCAGACTTACCGAGSpASpG^spA -28 1493,5 1820,8 - 1836,8 - 45 HLAB 30210 2ff9 ACACRGACTTACCGAGSpASpG^spA -28 1493,5 1820,8 - 1836,8 - 46 HLAB 3621 2f20 CGGGTCTCACACCCTCCSpASpG^spA -28 1493,5 1820,8 - 1836,8 - 47 HLAB 3622 2f20 CGGGTCTCACACCCTCCSPASPG^spA -28 1413,4 1756,7 - 47 HLAB 3622 2f20 CGGGTCTCACACCCTCCSPASPG^spA -14 1467,4 1794,7 1770,6 1810,7 1785,6 48 HLAB 3623 2f20 CGGGTCTCACACCCTCCSPASPG^spA -14 1467,4 1794,7 1770,6 1810,7 1785,6 49 HLAB 3624 2f20 CGGGTCTCACACCCTCCSPASPG^spA -14 1467,4 1794,7 1770,6 1810,7 1785,6 50 HLAB 3625 2f20 CGGGTCTCACACTTGGCSPASPG^spA -14 1467,4 1794,7 1770,6 1810,7 1785,6 50 HLAB 3631 1r20 CCCASGTCGCAGCCGTACSPASPG^spT -28 1085,1 - 1388,3 1428,4 1403,3 51 HLAB 3632 1r20 CCCASGTCGCAGCCGTACSPASPT -28 1085,1 - 1388,3 1428,4 1403,3			ACACAGACTTACCGAGspAspG^spG					-	
40         HLAB 3025 2ff9         ACACAGACTTACCGAGSpTspG^spA         -28         1481,4         1811,7         -         1827,7         -           41         HLAB 3026 2ff9         RCACAGACTGACCGAGSpTspG^spA         -28         1481,4         1811,7         -         1827,7         -           42         HLAB 3027 2ff9         ACACAGGCTGACCGAGSpASpG^spA         -28         1493,5         1820,8         -         1836,8         -           43         HLAB 3029 2ff9         RCACAGACTTACCGAGSpASpG^spA         -28         1493,5         1820,8         -         1836,8         -           45         HLAB 30210 2ff9         ACACRGACTTACCGAGSPASPG^spA         -28         1493,5         1820,8         -         1836,8         -           45         HLAB 30210 2ff9         ACACRGACTTACCGAGSPASPG^spA         -28         1493,5         1820,8         -         1836,8         -           46         HLAB 30210 2ff9         ACACRGACTTACCGAGSPASPG^spA         -28         1493,5         1820,8         -         1836,8         -           47         HLAB 3621 2f20         CGGGTCTCACACCCTCCSPASPG^spA         -28         1413,4         -         -         1756,7         -           48         HLAB 3623 2f20         CGGG	38	HLAB_3023_2119	RCACAGACTGACCGAGSpAspG^spG				<u> </u>		
41         HLAB_3026_2ff9         RCACAGACTGACCGAGSpTspG^spA         -28         1481,4         1811,7         -         1827,7         -           42         HLAB_3027_2ff9         ACACAGGCTGACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           43         HLAB_3028_2ff9         RCACAGACTGACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           44         HLAB_3029_2ff9         GCRCAGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           45         HLAB_30210_2ff9         ACACRGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           46         HLAB_3021_2f20         CGGGTCTCACACCCTCCspAspG^spA         -28         1413,4         -         -         1756,7         -           47         HLAB_3622_2f20         CGGGTCTCACACCCTCCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           49         HLAB_3622_2f20         CGGGTCTCACACTCTGCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           50         HLAB_3622_2f20 </td <td></td> <td>HLAB 3024 2119</td> <td>GCACAGACTGGCCGAGSp1spG^spA</td> <td></td> <td></td> <td></td> <td><u> </u></td> <td></td> <td>-</td>		HLAB 3024 2119	GCACAGACTGGCCGAGSp1spG^spA				<u> </u>		-
42         HLAB 3027 2f19         ACACAGGCTGACCGAGSpAspG^spA         -28         1493,5         1820,8         - 1836,8         -           43         HLAB 3028 2f19         RCACAGACTGACCGAGSpAspG^spA         -28         1493,5         1820,8         - 1836,8         -           44         HLAB 3029 2f19         GCRCAGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         - 1836,8         -           45         HLAB 30210 2f19         ACACRGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         - 1836,8         -           46         HLAB 3621 2f20         CGGGTCTCACACCCTCCSpAspG^spA         -28         1413,4         1756,7         -           47         HLAB 3622 2f20         CGGGTCTCACACCCTCCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           48         HLAB 3623 2f20         CGGGTCTCACACTTGGCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           50         HLAB 3625 2f20         CGGGTCTCACACTCTCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           51         HLAB 3626 2f20         CGGGTCTCACACTCTCSpAspG^spG         -14         1483,4									
43         HLAB         3028         2f19         RCACAGACTGACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           44         HLAB         3029         2f19         GCRCAGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           45         HLAB         30210         2f19         ACACRGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           46         HLAB         3621         2f20         CGGGTCTCACACCCTCCspAspC^spA         -28         1413,4         -         -         1756,7         -           47         HLAB         3622         2f20         CGGGTCTCACACCCTCCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           48         HLAB         3623         2f20         CGGGTCTCACACCTTCGspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           49         HLAB         3624         2f20         CGGGTCTCACACTTGCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           50         HLAB									
44         HLAB_3029_2f19         GCRCAGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           45         HLAB_30210_2f19         ACACRGACTTACCGAGSpAspG^spA         -28         1493,5         1820,8         -         1836,8         -           46         HLAB_3621_2f20         CGGGTCTCACACCCTCCSpAspC^spA         -28         1413,4         -         -         1756,7         -           47         HLAB_3622_2f20         CGGGTCTCACACYCATCCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           48         HLAB_3623_2f20         CGGGTCTCACACCCTCCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           49         HLAB_3624_2f20         CGGGTCTCACACTTGGCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           50         HLAB_3625_2f20         CGGGTCTCACACTCCTCSpAspG^spG         -14         1483,4         -         -         -         1801,6           51         HLAB_3631_1r20         CCCASGTCGCAGCCGTACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3           52         HLAB									
45         HLAB_30210_2f19         ACACRGACTTACCGAGspAspG^spA         -28         1493,5         1820,8         -         1836,8         -           46         HLAB_3621_2f20         CGGGTCTCACACCCTCCspAspC^spA         -28         1413,4         -         -         1756,7         -           47         HLAB_3622_2f20         CGGGTCTCACACCCTCCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           48         HLAB_3623_2f20         CGGGTCTCACACCTCCSpAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           49         HLAB_3624_2f20         CGGGTCTCACACTTGGCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           50         HLAB_3625_2f20         CGGGTCTCACACTCTCSpAspG^spG         -14         1483,4         -         -         -         1801,6           51         HLAB_3626_2f20         CGGGTCTCACACCCTCCspAspG^spT         0         1472,4         -         -         1815,7         -           52         HLAB_3631_1r20         CCCASGTCGCAGCCGTACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3           53         HLAB_3632_1r2	<del>10</del>	HI AR 2020 2119	GCPCAGACTTACCGAGSPASPG SPA						
46 HLAB 3621_2f20 CGGGTCTCACACCCTCCspAspC^spA -28 1413,4 1756,7 - 47 HLAB 3622_2f20 CGGGTCTCACAYCATCCspAspG^spA -14 1467,4 1794,7 1770,6 1810,7 1785,6 48 HLAB 3623_2f20 CGGKTCTCACACCCTCCspAspG^spA -14 1467,4 1794,7 1770,6 1810,7 1785,6 49 HLAB 3624_2f20 CGGGTCTCACACTTGGCspAspG^spA -14 1467,4 1794,7 1770,6 1810,7 1785,6 50 HLAB 3625_2f20 CGGGTCTCACACTCATCCspAspG^spA -14 1483,4 1801,6 51 HLAB 3626_2f20 CGGGTCTCACACCCTCCspAspG^spT 0 1472,4 1815,7 - 52 HLAB 3631_1r20 CCCASGTCGCAGCCGTACspA^spT -28 1085,1 - 1388,3 1428,4 1403,3 53 HLAB 3632_1r20 CCCABGTCGCAGCCATACspA^spT -28 1085,1 - 1388,3 1428,4 1403,3	45	HI AR 30240 2619	ACACRGACTTACCGAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAG						
47       HLAB 3622 2f20       CGGGTCTCACAYCATCCspAspG^spA       -14       1467,4       1794,7       1770,6       1810,7       1785,6         48       HLAB 3623 2f20       CGGKTCTCACACCCTCCspAspG^spA       -14       1467,4       1794,7       1770,6       1810,7       1785,6         49       HLAB 3624 2f20       CGGGTCTCACACTTGGCspAspG^spA       -14       1467,4       1794,7       1770,6       1810,7       1785,6         50       HLAB 3625 2f20       CGGGTCTCACACTCATCCSpAspG^spG       -14       1483,4       -       -       -       1801,6         51       HLAB 3632 2f20       CGGGTCTCACACCCTCCspAspG^spT       0       1472,4       -       -       1815,7       -         52       HLAB 3631 1r20       CCCASGTCGCAGCCGTACspA^spT       -28       1085,1       -       1388,3       1428,4       1403,3         53       HLAB 3632 1r20       CCCABGTCGCAGCCATACspA^spT       -28       1085,1       -       1388,3       1428,4       1403,3	H	77245_00210_2113	лолополот глосолозразро эрд	-20	1433,3	1020,0		1830,8	
47         HLAB 3622 2f20         CGGGTCTCACAYCATCCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           48         HLAB 3623 2f20         CGGKTCTCACACCCTCCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           49         HLAB 3624 2f20         CGGGTCTCACACTTGGCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           50         HLAB 3625 2f20         CGGGTCTCACACTCATCCSpAspG^spA         -14         1483,4         -         -         -         1801,6           51         HLAB 3626 2f20         CGGGTCTCACACCCTCCSpAspG^spT         0         1472,4         -         -         1815,7         -           52         HLAB 3631 1r20         CCCASGTCGCAGCCGTACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3           53         HLAB 3632 1r20         CCCABGTCGCAGCCATACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3	46	HLAB_3621_2f20	CGGGTCTCACACCCTCCspAspC^spA	-28	1413.4			1756.7	
48         HLAB 3623 2f20         CGGKTCTCACACCCTCCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           49         HLAB 3624 2f20         CGGGTCTCACACTTGGCspAspG^spA         -14         1467,4         1794,7         1770,6         1810,7         1785,6           50         HLAB 3625 2f20         CGGGTCTCACATCATCCspAspG^spG         -14         1483,4         -         -         1801,6           51         HLAB 3626 2f20         CGGGTCTCACACCCTCCspAspG^spT         0         1472,4         -         -         1815,7         -           52         HLAB 3631 1r20         CCCASGTCGCAGCCGTACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3           53         HLAB 3632 1r20         CCCABGTCGCAGCCATACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3		HLAB 3622 2f20	CGGGTCTCACAYCATCCspAspG^spA						
49       HLAB 3624 2f20       CGGGTCTCACACTTGGCspAspG^spA       -14       1467,4       1794,7       1770,6       1810,7       1785,6         50       HLAB 3625 2f20       CGGGTCTCACATCATCCspAspG^spG       -14       1483,4       -       -       -       1801,6         51       HLAB 3626 2f20       CGGGTCTCACACCCTCCspAspG^spT       0       1472,4       -       -       1815,7       -         52       HLAB 3631 1r20       CCCASGTCGCAGCCGTACspA^spT       -28       1085,1       -       1388,3       1428,4       1403,3         53       HLAB 3632 1r20       CCCABGTCGCAGCCATACspA^spT       -28       1085,1       -       1388,3       1428,4       1403,3	48	HLAB 3623 2f20	CGGKTCTCACACCCTCCspAspG^spA						
50         HLAB_3625_2f20         CGGGTCTCACATCATCCspAspG^spG         -14         1483,4         -         -         -         1801,6           51         HLAB_3626_2f20         CGGGTCTCACACCCTCCspAspG^spT         0         1472,4         -         -         1815,7         -           52         HLAB_3631_1r20         CCCASGTCGCAGCCGTACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3           53         HLAB_3632_1r20         CCCABGTCGCAGCCATACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3	49	HLAB 3624 2f20	CGGGTCTCACACTTGGCspAspG^spA						
51         HLAB 3626 2f20         CGGGTCTCACACCCTCCspAspG^spT         0         1472,4         -         -         1815,7         -           52         HLAB 3631 1r20         CCCASGTCGCAGCCGTACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3           53         HLAB 3632 1r20         CCCABGTCGCAGCCATACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3	_	HLAB_3625_2f20	CGGGTCTCACATCATCCspAspG^spG						
52         HLAB_3631_1r20         CCCASGTCGCAGCCGTACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3           53         HLAB_3632_1r20         CCCABGTCGCAGCCATACspA^spT         -28         1085,1         -         1388,3         1428,4         1403,3	51	HLAB 3626 2f20	CGGGTCTCACACCCTCCspAspG^spT						
53 HLAB_3632_1r20 CCCABGTCGCAGCCATACspA^spT -28 1085,1 - 1388,3 1428,4 1403,3	<b> </b>								
53 HLAB_3632_1r20 CCCABGTCGCAGCCATACspA^spT -28 1085,1 - 1388,3 1428,4 1403,3	52	HLAB_3631_1r20	CCCASGTCGCAGCCGTACspA^spT	-28	1085,1	-	1388.3	1428,4	1403,3
	53	HLAB_3632_1r20	CCCABGTCGCAGCCATACspA^spT						
	54	HLAB_3633_1r20	CCCASGTCGCAGCCAAACspA^spT	-28		-			

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	55		CCCACGTCGCAGCCAGACspA^spT	-28	1085,1	-	1388,3		140:
	56		CCCACGTCGCAGCCGCACspA^spT	-28	1085,1	-	1388,3	1428,4	140:
	57		CCCACGTCGCAGCCTTACspA^spT	-28	1085,1	•	1388,3	1428,4	1400
	58	HLAB_3637_1r20	CCCACGTCGCAGCCGTACspG^spT	0	1129,1	•	1432,3	1472,4	1447
	59		TCCGGCCCCAKGTCGCAGspC^spC	0	1114,1	1441,4	•	1457,4	1432
	60	HLAB_3692_1f20	TCGGGCCCCASGTCGCAGspC^spC	0	1114,1	1441,4	•	1457,4	1432
	55		GGCGCCTCCTCCGCGGGspTspA^spC	-28	1444,4	-	1747,6	•	•
	56		GGCGCCTCCTCCSCGGGspCspA^spT	. 0	1472,4	1799,7	•	1815,7	-
5	57		GGCGCYTCCTCCGCGGGspCspA^spT	0	1472,4	1799,7	•	1815,7	•
3	58		GGCGTCTCCTCCGCGGTspTspA^spT	0	1462,4	•	1765,6		•
	59	HLAB_4125_2f20	GGCGCCTCCTCCGCGGGspTspA^spT	-14	1473,4	-	1776,6	•	•
	60		TCCTCCGCGGGTATGAAspCspA^spG	0	1481,4	1808,7	•	•	•
	61		TCCTCCACGGGTACCACspCspA^spG	0	1457,4	-	•	8	1775
	62		TCCTGCGCGGGTACCACspCspA^spG	0	1457,4	-	-	•	1775
	63		TCCTCCGCGGGTACCACspCspA^spG	0	1457,4		•	•	1775
	64	HLAB_4185_2f20	TCCTCTGCGGGTACCACspCspA^spG	0	1457,4	•		1	1775
	65		TCCTCCGCGGGTACCAGspCspA^spG	0	1497,4	1824,7	1800,6	1840,7	1815
10	66		TMCTCCGCGGGTACCGGspCspA^spG	0	1497,4	1824,7	1800,6	1840,7	1815
	67	HLAB_4188_2f20	TCCTCCGCGGGTACCAGspCspG^spG	0	1513,4	-	•	1856,7	•
	68	HLAB_4191_2r20	AATCCTTGCCGTCGTAGspGspC^spT	-14	1474,4	1801,7	•		•
	69		AATCCTTGCCGTCGTAGspGspC^spA	-28	1469,4	•	•	1812,7	-
	70		AATTCTTGCCGTCGTAGspGspC^spG	0	1513,4	1840,7	•	1856,7	1831
	71		AATCTTTGCCGTCGTAGspGspC^spG	0	1513,4	1840,7	•	1856,7	1831
	72	HLAB_4195_2r20	AATCCTTGCCGTCGYAGspGspC^spG	0	1513,4	1840,7		1856,7	1831
10			TCMTTCAGGGCGATGTAAspT^spC	-14	1201,3	•	1504,4	-	1519
15	74	HLAB_4352n_1r20	TCGTTCAGGGCGATGTAAspT^spT	0	1230,3	-	1533,5	-	-
	75	HLAB_5271_1f20	CAAGTGGGAGGCGGCCCTspT^spG	0	1246,3	-			1564
	76	HLAB_5272_1120	CAAGTKGGAGGCGGCCCGspT^spG	0	1271,3	1598,6	1574,3	-	1589
		111 472 5004 4500	000000000000000000000000000000000000000	<u>                                     </u>					
_	77		GGCCCGTGYGGCGGAGCAspG^spC	0	1138,1			1481,3	1456
-	78 79	HLAB 5392 1120	GGCCCGTGTCGCGGAGCAspG^spG	0	1178,1	1505,4	•		-
	80		GGCCGTGWGGCGGAGCAspG^spG	Ö	1178,1	1505,4		4400	
ļ	80	TLAD 0334 1120	GGCCCGTGAGGCGGAGCAspG^spT	0	1153,1			1496,4	=
20	81	UI AR EEGA 4-20	GCGGAGCGACTCCACGCAspC^spT	<del> </del>	44404			4455	
	82	LILAD SSST 1120	GCGGAGCCACTCCACGCAspC*spT GCGGAGCCACTCCACGCAspC*spT	00	1113,1			1456,4	
	83	UI AD 5502 1:20	GCGGAGCCACTCCACGCASpC*spT GCGGAGCCAATCCACGCASpC*spT		1113,1	-		1456,4	
	84	HI AR EEGA 4-20	GCGGAGCCAATCCACGCAspC*spT GCGGAGCCACTCCACGCAspC*spG	0	1113,1			1456,4	4470
			GCGGAGCGACTCCACGCAspC*spG GCGGAGCGACTCCRCGCAspC*spA	0	1152,1 1122,1	4440.4	4405.2		1470
	88	LI AR REGE 4-20	GCGGAGCSACTCCACGCASpC*SpA GCGGAGCSACTCCACGCASpC*SpA						
	87	HI AR 5507 1-20	GCGGAGCCACTCCACGCAspC^spA GCGGAGCCCGTCCACGCAspC^spA	-14	1122,1	1449,1	1425,3		
	<del>اٽ'</del> ا	11LMD_0001_1120	- SPA	-14	1122,1	1449,1	1425,3		
	88	HI AR 5744 4-20	CTCCAGGTAYCTGCGGAGspC^spG	<u> </u>	44544	4404 4			
25	89	HI AR: 5712 1:20	CTCCAGGTATCTGCGGAGSpC*spG CTCCAGGTRTCTGCGGAGSpC*spC	0	1154,1	1481,4	44473		
د بیر	<del>  "</del>	11LAD 0/ 14_1120	O TOUMOGIN TO TOUGOMOSPU SPC	۱ ۷	1114,1	1441,4	1417,3		
	90	HI AR 583 1r19	ACCTGGAGAACGGGAAGspG^spA		4479 4	4505.4		1521,4	
ŀ		11270,000,1113	Noor govovvoggovvosho, shy	0	1178,1	1505,4		1041,4	

TABLE VI

No   Name   Sequence   CT   Primer   A   C   G	1431,
1 DRB1_1251_1r20 CATTGAAGAAATGACACTspC^spC 0 1098,1 - 1392,3 - 2 DRB1_1252_1r20 CGTTGAAGAAATGACACTspT^spA 0 1230,1 3 DRB1_1253_1r20 CATTGAAGAAATGACACTspC^spA 0 1113,1 1440,4 1416,3 1456,4 DRB1_1254_1r20 CATTGAAGAAWTAACACTspC^spA 0 1113,2 1440,4 1416,3 1456,5 DRB1_1255_1r20 CRTTGAAGAAWTAACACTspC^spA 0 1113,3 1440,4 1416,3 1456,5 DRB1_1255_1r20 CRTTGAAGAAATGACACTspC^spA 0 1113,3 1440,4 1416,3 1456,5 DRB1_1961_1f19 CATCTATAACCAAGAGGspA^spA 0 1162,f DRB1_1962_1f19 CTTCTATCACCAAGAGGspA^spG 0 1178,1 1505,4 DRB1_1963_1f19 CTTCTATAATCARGAGGspA^spG 0 1178,1 1505,4 DRB1_1964_1f19 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 DRB1_1965_1f19 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4	1548, 1431, 1431, 1431, 1480, 1496, 1496, 1496, 1496,
2 DRB1_1252_1r20 CGTTGAAGAAATGACACTspT^spA 0 1230,1	1431, 1431, 1431, 1480, 1496, 1496, 1496, 1496,
3 DRB1_1253_1r20 CATTGAAGAAATGACATTspC^spA 0 1113,1 1440,4 1416,3 1456 4 DRB1_1254_1r20 CATTGAAGAAWTAACACTspC^spA 0 1113,2 1440,4 1416,3 1456 5 DRB1_1255_1r20 CRTTGAAGAAATGACACTspC^spA 0 1113,3 1440,4 1416,3 1456 5 DRB1_1961_1f19 CATCTATAACCAAGAGGspA^spA 0 1162,1 7 DRB1_1962_1f19 CTTCTATCACCAAGAGGspA^spG 0 1178,1 1505,4 8 DRB1_1963_1f19 CTTCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 9 DRB1_1964_1f19 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1965_1f19 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 11 DRB1_1966_1f19 CTTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 12 DRB1_1967_1f19 CTTCGATAACCAGGAGGspA^spG 0 1178,1 1505,4 13 DRB1_1968_1f19 CTTCTATAACCAGGAGGspA^spG 0 1178,1 1505,4 14 DRB1_1968_1f19 CTTCTATAACCTGGAGGspA^spG 0 1178,1 1505,4	1431, 1431, 1431, 1480, 1496, 1496, 1496, 1496,
4 DRB1_1254_1r20 CATTGAAGAAWTAACACTspC^spA 0 1113,2 1440,4 1416,3 1456, 5 DRB1_1255_1r20 CRTTGAAGAAATGACACTspC^spA 0 1113,3 1440,4 1416,3 1456, 6 DRB1_1961_1f19 CATCTATAACCAAGAGGspA^spA 0 1162,1 7 DRB1_1962_1f19 CTTCTATCACCAAGARGspA^spG 0 1178,1 1505,4 8 DRB1_1963_1f19 CTTCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 9 DRB1_1964_1f19 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1965_1f19 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 11 DRB1_1966_1f19 CTTCCATAACCAGGAGGspA^spG 0 1178,1 1505,4 12 DRB1_1967_1f19 CTTCGATAACCAGGAGGspA^spG 0 1178,1 1505,4 13 DRB1_1968_1f19 CTTCTATAACCAGGAGGspA^spG 0 1178,1 1505,4 14 DRB1_1968_1f19 CTTCTATAACCTGGAGGspA^spG 0 1178,1 1505,4	1431, 1431, 1480, 1496, 1496, 1496, 1496,
5 DRB1_1255_1r20 CRTTGAAGAAATGACACTspC^spA 0 1113,3 1440,4 1416,3 1456  6 DRB1_1961_1ff9 CATCTATAACCAAGAGGspA^spA 0 1162,1  7 DRB1_1962_1ff9 CTTCTATCACCAAGARGspA^spG 0 1178,1 1505,4  8 DRB1_1963_1ff9 CTTCTATAATCARGAGGspA^spG 0 1178,1 1505,4  9 DRB1_1964_1ff9 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4  10 DRB1_1965_1ff9 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4  11 DRB1_1966_1ff9 CTTCCATAACCAGGAGGspA^spG 0 1178,1 1505,4  12 DRB1_1967_1ff9 CTTCGATAACCAGGAGGspA^spG 0 1178,1 1505,4  13 DRB1_1968_1ff9 CTTCTATAACCTGGAGGspA^spG 0 1178,1 1505,4  14 DRB1_1971_1r20 CGTCGCTGTCGAAGCGCAspG^spG 0 1178,1 1505,4	1431, 1480, 1496, 1496, 1496, 1496,
5 6 DRB1_1961_1ff9 CATCTATAACCAAGAGGspA^spA 0 1162,f	1480, 1496, 1496, 1496, 1496,
7 DRB1_1962_1ff9 CTTCTATCACCAAGARGspA^spG 0 1178,1 1505,4 8 DRB1_1963_1ff9 CTTCTATAATCARGAGGspA^spG 0 1178,1 1505,4 9 DRB1_1964_1ff9 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1965_1ff9 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 11 DRB1_1966_1ff9 CTTCCATAACCAGGAGGspA^spG 0 1178,1 1505,4 12 DRB1_1967_1ff9 CTTCGATAACCAGGAGGspA^spG 0 1178,1 1505,4 13 DRB1_1968_1ff9 CTTCTATAACCTGGAGGspA^spG 0 1178,1 1505,4 14 DRB1_1971_1r20 CGTCGCTGTCGAAGCGCAspG^spG 0 1178,1 1505,4	1496, 1496, 1496, 1496,
7 DRB1_1962_1ff9 CTTCTATCACCAAGARGspA^spG 0 1178,1 1505,4 8 DRB1_1963_1ff9 CTTCTATAATCARGAGGspA^spG 0 1178,1 1505,4 9 DRB1_1964_1ff9 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1965_1ff9 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 11 DRB1_1966_1ff9 CTTCCATAACCAGGAGGspA^spG 0 1178,1 1505,4 12 DRB1_1967_1ff9 CTTCGATAACCAGGAGGspA^spG 0 1178,1 1505,4 13 DRB1_1968_1ff9 CTTCTATAACCTGGAGGspA^spG 0 1178,1 1505,4 14 DRB1_1971_1r20 CGTCGCTGTCGAAGCGCAspG^spG 0 1178,1 1505,4	1496, 1496, 1496, 1496,
8 DRB1_1963_1ff9 CTTCTATAATCARGAGGspA^spG 0 1178,1 1505,4 9 DRB1_1964_1ff9 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1965_1ff9 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 11 DRB1_1966_1ff9 CTTCCATAACCAGGAGGspA^spG 0 1178,1 1505,4 12 DRB1_1967_1ff9 CTTCGATAACCAGGAGGspA^spG 0 1178,1 1505,4 13 DRB1_1968_1ff9 CTTCTATAACCTGGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1971_1r20 CGTCGCTGTCGAAGCGCAspG^spG 0 1178,1 1505,4	1496, 1496, 1496, 1496,
9 DRB1_1964_1ff9 CGTCCATAACCAAGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1965_1ff9 CATCTATAACCAAGAGGspA^spG 0 1178,1 1505,4 11 DRB1_1966_1ff9 CTTCCATAACCRGGAGGspA^spG 0 1178,1 1505,4 12 DRB1_1967_1ff9 CTTCGATAACCAGGAGGspA^spG 0 1178,1 1505,4 13 DRB1_1968_1ff9 CTTCTATAACCTGGAGGspA^spG 0 1178,1 1505,4 10 DRB1_1971_1r20 CGTCGCTGTCGAAGCGCAspG^spG 0 1178,1 1505,4	1496, 1496, 1496,
10 DRB1_1965_1ff9 CATCTATAACCAAGAGGspA^spG	1496, 1496,
11 DRB1_1966_1f19 CTTCCATAACCRGGAGGspA^spG	1496,
12 DRB1_1967_1f19 CTTCGATAACCAGGAGGspA^spG	
13 DRB1_1968_1f19 CTTCTATAACCTGGAGGspA^spG	1496.
10 14 DRB1_1971_1r20 CGTCGCTGTCGAAGCGCAspG^spG 0 1178,1 1505,4	
11 21.21_101 _1120   0010001010074000075p0 Sp0	1496,
11 21.21_101 _1120   0010001010074000075p0 Sp0	
I 4E I DDD4 4079 4-90 ICCTCCCTCTCCTCCCCCCCCCCCCCCCCCCCCCCCCC	1496,
15 DRB1_1972_1r20 CGTCGCTGTCGTAGCGCGspC^spG 0 1154,1	1472,:
16 DRB1_1973_1r20 CGTCGCTGTCGAAGCGCAspA^spG 0 1162,1	1480,:
17 DRB1_1974_1r20 CGTCGCTGTCGAAGYGCAspC^spG -28 1110,1 1437,4 - 1453,	1428,:
18 DRB1_1975_1r20 CGTCGCTGTCGAASCGCAspC^spG -28 1110,1 1437,4 - 1453,	1428,
19 DRB1_2271_1f20 CGACAGCGACGTGGGGGAspC^spT 0 1113,1 1440,4	
20 DRB1_2272_1f20 CGACAGCGACGTGVGGGAspG^spT	1471,
15	
21 DRB1_2611_1r20 TTCTGGCTGTTCCAGTACspT^spG	-
22 DRB1_2612_1r20 TTCTGGCTGTTCCAGTACspC^spC	-
23 DRB1_2613_1r20 TTCTGGCTGTTCCAGTAGspT^spC	
24 DRB1_2614_1r20 TTCTGGCTGTTCCAGTRCspT^spC -14 1177,2 1504,5 1480,4 1520,	<u> </u>
25 DRB1_2615_1r20 TTCYGGCTGTTCCAGGACspT^spC -14 1177,2 1504,5 1480,4 1520,	-
00 8004 4004 400 0000 0000 0000	
26 DRB1_2861_1f19 CTGGAACAGCCAGAAGAspA^spC -28 1122,1 1449,4	<u> </u>
======================================	1456,3
28 DRB1 2991 1f20 GAAGGACHTCCTGGAGCAsnGAsnG 0 1178 1 1481 3	
1401,3	<u> </u>
00 000 100,1	
04 5004 400 04400 04400 04400	-
31 DRB1_2994_1f20 GAAGGACYTCCTGGAAGASpC^spA -14 1108,1 1435,1 - 1453,	-
32 DRB1_2995_1f20 GAAGGACATCCTGGAGCAspG^spA	•
1021)	<u> </u>
34 DRB1_2997_1f20 GAAGGACHTCCTGGAAGASpC^spG 0 1138,1 1465,4	
25 35 DBR1 3081 1r20 GTCTGCAATAGGTGTCCAspC^spG 0 11381 - 14413	
35 DBN1_3061_1120 GTCTGCAATAGGTGTCCASpC^spG 0 1138,1 - 1441,3 -	
36 DRB1_3082_1r20 GTCTGCARTAGGCGTCCAspC^spC -14 1084,1 1411,4 1387,3 1427,4	1402,3
37 DRB1_3083_1r20 GTCTGCAGTAATTGTCCAspC^spC -14 1084,1 1411,4 1387,3 1427,4	1402,3
38 DRB1_3084_1r20 GTCTGCACACGGTGTCCAspC^spC -14 1084,1 1411,4 1387,3 1427,4 39 DRB1_3085_1r20_GTCTGCAGTAGGTGTCCAspC^spC -14 1084_1 1411,4 1387_3 1427,4 1411,4 1387_3 1427,4 1411,4 1387_3 1427,4 1411,4 1387_3 1427,4 1411,4 1387_3 1427,4 1411,4 1387_3 1427,4 1411,4 1411,4 1387_3 1427,4 1411	1402,3
40 0000 400 000000 1141,7 1001,0 1421,	1402,3
40 DRB1_3086_1r20 GTCTGCAATAGGTGTCCAspC^spC -14 1084,1 1411,4 1387,3 1427,4	1402,3
41 DRB1 341 1f19 TGCAGACACACTACSGspG^spG 0 11941 - 1497 3	
1707,3	1512,3
30 42 PPP4 2454 4 00 000700 107070 107070	
42 DRB1_3451_1r20 CGCTGCACTGTGAATCTCspT^spC	•
43 DRB1_3452_1r20 CTCTGCACTGTGAAGCTCspT^spC	-
44 DRB1_3453_1r20 CGCTGCACYGTGAAGCTCspT^spC 0 1191,3 1518,5 1494,4 -	-

The resolution achievable by 19 markers each for HLA-A and HLA-B and the ten markers for HLA-DRB1 are listed in Tables VII to IX below.

### TABLE VII

Frequent Alleles of	Group of	Rare Alleles with same Mini-	Resolution
HLA-A	frequent Alleles	Haplotype Profile	(in %)
	with same four-		
	digit type		
A*0101	NATURE.	A*0103, 20, 20, A*0109	98,3
	A*010102	Des Succes Chabers	
A*0201	a to 2010 311,	A*0204, A*0225, A*0225,	93,4
	A TOO O TO O	A*0231, A*0232N, A*0242,	
	A*020103, A*020104,	A*0260 A*0264	
	A-020104,	A*0260, A*0264, A*0266, A*0267	
•	A*020109	A 0207	
	A*020102		100
	A*020105		100
	A*020106		100
	A*020107		100
A*0301	AFTOROLOUGH,	A*0303N, A*0304, A*0305,	97,6
	A SECULO DE LA COMPANION DE LA	A*0306, A*0311N	
	A*030102		100
	A*030103		100
A*2301		A*2306, 22000, A*2308N	98,6
A*2402		A*2404, A 240 N, A 24 N,	94,5
	AZZOZOTEK,	A*2426, A*2427, A*2432,	
	A*240202,	A*2435, A*2436N, A*2437,	
		A*2439	
A #0000	A*240204		
A*2902	A*290201	A*2906, A*2908N	98,3
	A*290202	A 2700, A 2700N	100
A*3001	A*3001		100
A*3002	A*3002		100
** 0.402	112 3002	. <u></u>	100

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exons 2 and 3.

#### TABLE VIII

Frequent Alleles of HLA-B	Groups of frequent Alleles with same four-digit type	Rare Alleles with same Mini- Haplotype Profile	Resolution (in %)
B*0702	B*070201, B*070202, B*070203, B*070204	B*0703, B*0721, B*0722, B*0723, B*0730, B*0733, B*0735	98,0
B*0801	6330809	B*0808N, B*0818, BE0819N	99,3
B*1302	B*1302	B*1308	99,6
B*1501	B*150103, B*150104	B*1528, B*1533, B*1534, B*1560, B*1575, B*1578, B*1579N, B*1581, B*1582	97,6
741001	B*150102		100
B*1801	B*180102	B*1805, E 8 7/5	99,3
B*3501	B*350102	B*3507, B*3541, B*3541,	98,7
B*3503	B*3503	B*3536	99,6
B*4001		B*4011, B*401401, B*401402, B*401403, B*4022N	98,7
	B*400103		100
	B*400104	B*4004	99,6
B*4402	B*440203	B*4411, B*4422, B*4423N, B*4433, B*4434, B*4435	97,8
B*4403	B*440301	B*4413, B*4426, B*4429, B*4430, B*4432, B*4436, B*4437, B*4438, B*4439	98,2
74.04	B*440302	B*4407	99,6
B*5101	B*510102,	B*5112, B*5114, B*5118, B*5126, B*5127N, B*5128, B*5133	97,6
•	B*510103	•	100
74	B*510104	B*5124	99,6
B*5701	B*570101	B*5706, B*5708	99,5
	B*570102		100

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exons 2 and 3.



TABLE IX

Frequent Alleles of	Groups of frequent	Rare Alleles with same Mini-	Resolution
HLA-DRB1*	Alleles with same	Haplotype Profile	(in %)
	four-digit type		<u> </u>
DRB1*0101	DRB1*010101	DRB1*0105, DRB1*0107,	98,9
		DRB1*0111	
	DRB1*010102		100
DRB1*0301	DRB1*030101,	DRB1*0307, DRB1*0312,	97,2
	DRB1*030102	DRB1*0313, DRB1*0315,	
		DRB1*0316, DRB1*0318,	
		DRB1*0322, DRB1*0323	
DRB1*0401	DRB1*040101,	DRB1*0409, DRB1*0426,	98,6
	DRB1*040102	DRB1*0433	
DRB1*0701	DRB1*070101,	DRB1*0703, DRB1*0704,	98,3
	DRB1*070102	DRB1*0705, DRB1*0707	İ
DRB1*1101	Diggenstation out out	DRB1*112701,	97,5
	DRIED KNOP	DRB1*112702, DRB1*1130,	ł
•	DRB1*110103,	DRB1*1139	
<u>.</u>	DRB1*110104,		
	DRB1*110105		
DRB1*1104		DRB1*1134, DRB1*1146	98,9
DRB1*1302	DRB1*130201,	DRB1*1331, DRB1*1339,	98,6
	DRB1*130202	DRB1*1341	
DRB1*1501	DRB1*150101,	DRB1*1503, DRB1*1506,	98,0
	DRB1*150103,	DRB1*1509, DRB1*1513	
	DRB1*150105		
	DRB1*150102		100
	DRB1*150104	DRB1*1512	99,4

Capture: Alleles in a same field have the same mini-haplotype profile; grey high lighted are all alleles with identical sequences over exon 2 (base 101 to 356)



The complete list of HLA alleles and sub-groups generated by the most informative mini-haplotyping markers (ten each for HLA-A, HLA-B and HLA-DRB1) are listed in Tables X to XII below.

TABLE X

		_	_											•			• .	"															
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	A*6806	17 0	> т	<b>3</b> 7	G	A	A R	G	T	c	c	A	G G	G	A	e g	Т	G	T		G	G A	A	C	т		в	в	C	c	G	T	
	A*0244	17 9	· · · · · · · · · · · · · · · · · · ·	S C	C	Α	A A		c E	c	¢	A	90	G	A	G	T	G	T		G	G G	۸	C	Т		G	T	c	c	G	T	
	A*0254 A*0205	7 6		C	c	A	图 ^	-	C	C		A .			A .	C	Ţ	G	T		G	G G	1	C	Т		G	T	C	C	G	T	
	A*0208	Τ .	1	c	c	A S			T	C	C	A	G G	G	A	G	T	G	T			6 6 6 6	1	c	T SE		G	T ~	c	CE	S C	T -	
	A*6815	17 6	: T	c	c	A	A S	G	Т	c	G	A	G	G	A	G	7	G	· T			G A		c	T 188		G	T G	c	c	G	T	
	A*6802 A*6818N	7 6		o c	C	A		G	T	ç	C	A	G G	G	٨	6 8	Ţ	G.	Ţ			G A	٨	ç	I	2	G	G	ç	c	G	Ţ	
5	A*0228	7 0		c	c	A	M A	G	т <b>2</b>	c	c	A	3 0	G	A	G	T	G	· T		4.0	G A G G	1	c	T		0	G T	c	C	G	T	
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	A*0251 A*0261	T	- 2	2	c	A 9	<b>2</b> ^	G	T	C	¢	A	G	G	A	c Se	T	G	Ť		G	GG	^	č	Ť		G	Ť		č	6	Ť	ì
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	A*2504 A*2506	7 8		C	G	A		G	C	ç	C	AAA	6	G		e di	Ţ	G	T			G A	ļ	c	I		G	G		c	G	Ţ	
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	A*6602	T C	7	c	c	A \$	<b>A</b>	в	т 📆	c	c	A	a	G			T	G	T		G (	3 A	Â	C	T			-	C (		G		G G
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	A*1103 A*1104	ТС	_			6		G	c	c	c	A SE	翻	G	A (	3	т	G	T \$		A 6	_	^	c	-		G (	_			G		G G
	A*1107 A*110101	T C	TE	CCC		000			CCC	d C C		A REA	900	8	A (				T		A	6 G	A A	C	T		G	G	C		G	T	Ğ
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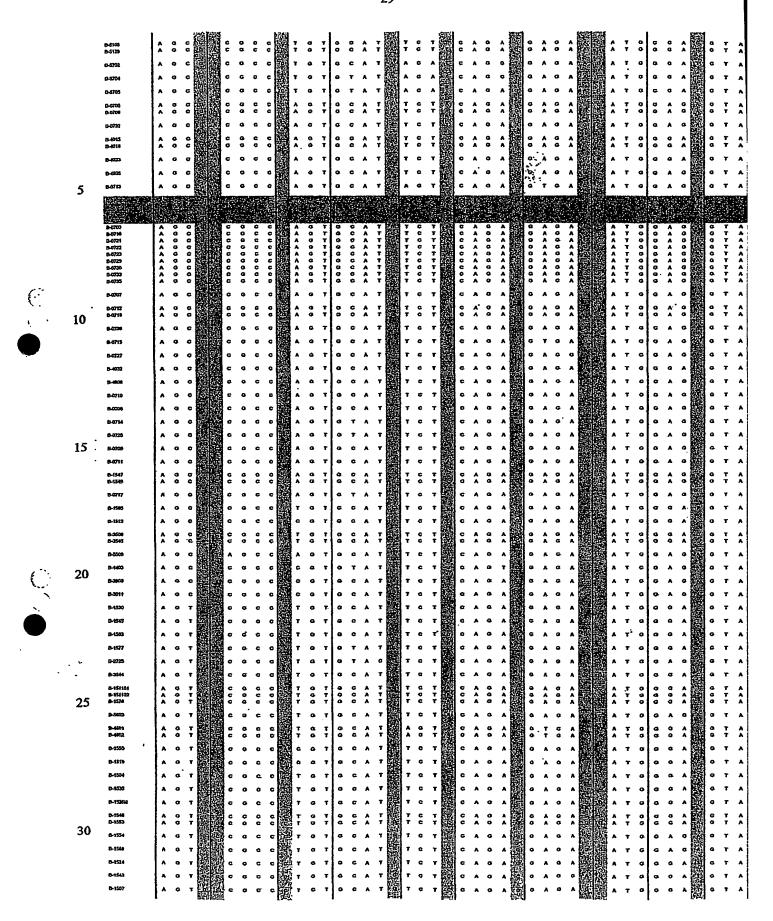
5	A*6810 A*6814 A*6808 A*0312 A*3402 A*3403 A*3404 A*3107 A*3107 A*3108 A*3105 A*310102 A*3109 A*330301 A*3301 A*	TOT		G G G G AA A AAAAAAAA	AA A AA AA AA AAAAAAAA A	T C T			A A A					6 6 6 6 6 6 6 6 6	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	AA G A GG G AA, A AA GGGGGGG T	000000000000000000000000000000000000000	GG A G AA A GG G GG AAAAAAA A	C C C C C T TT TTTTTT	TT T T T T T T T TTTTTTT				0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	00 6 6 66 666666	1 TELEVISION OF STREET STREET STREET
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15	A*3104 A*3004 A*3006 A*3010 A*3007	T C T T C T T C T T C T	C C C C T G T G T G T G T G T G T G T G	G A A A A A	A G A G A G A G A G				AA AA A AAA		AA			60 60 6	TT TT C T TT	** ** * * ***	G	AA AA A A A A A A A A A A A A A A A A	Ť	TT TT T.	000000000000000000000000000000000000000	9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9		000 00 00 000	<b>G</b> 1	
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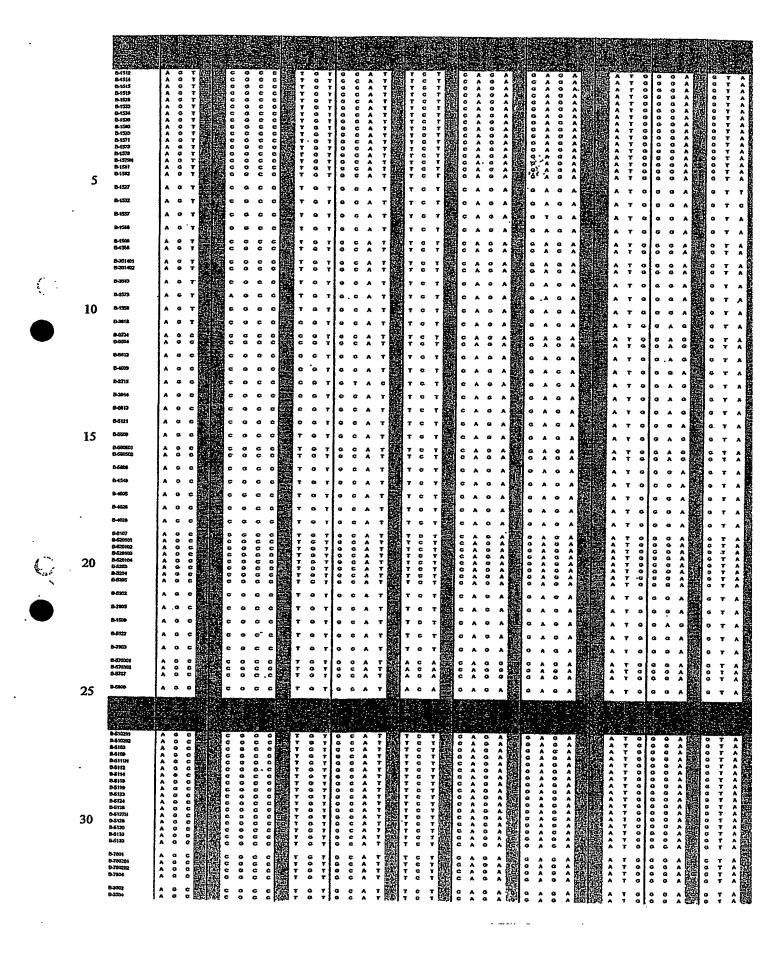
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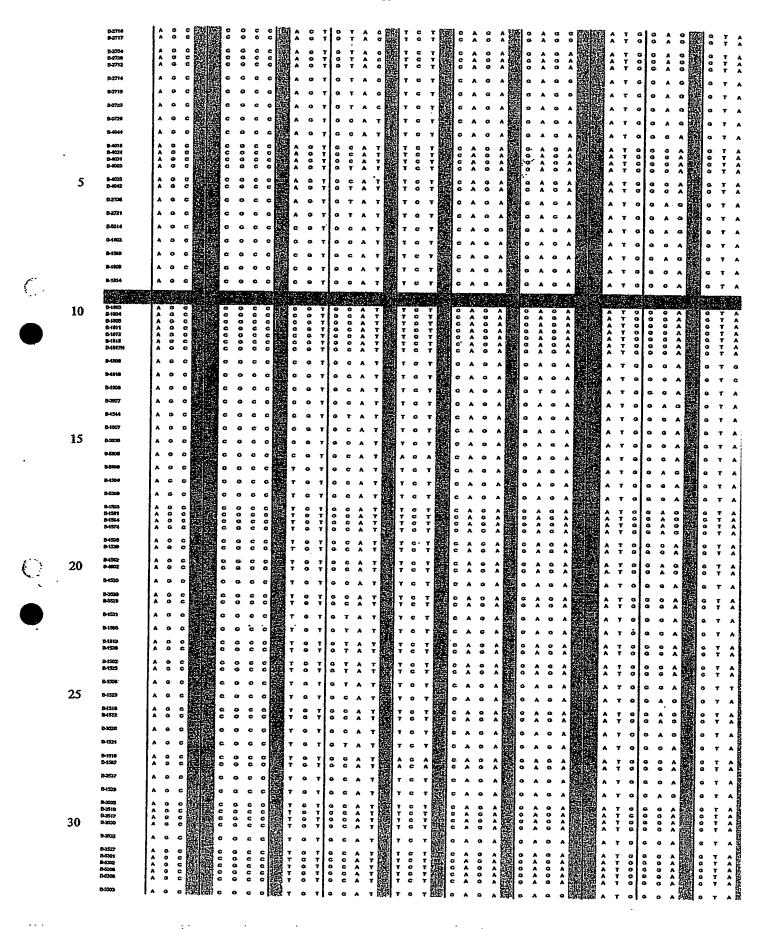
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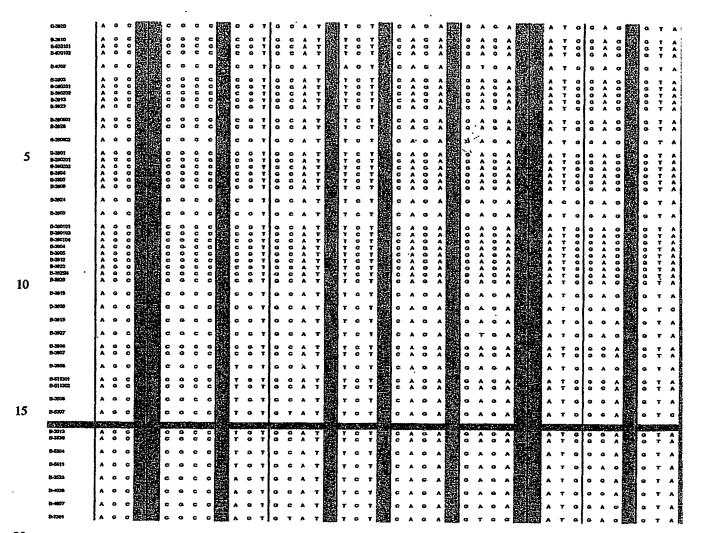




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# TABLE XII

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General strategy for medium resolution typing is described below:

For medium resolution typing a maximally informative set of marker positions were determined. These consist of positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81, 268, 559, 92, 123 and 396 of HLA-A (numbering starts at the transcription start position of exon 1), positions 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 of HLA-B (numbering starts at the transcription start position of exon 1), and positions 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 of HLA-DRB1 (numbering starts at the transcription start position of exon 1).

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In general, the order of the positions is from the most informative to the least informative with respect to the selection criteria of frequent and rare HLA alleles (see list of frequent HLA alleles above). Thus the ten markers (HLA-A and HLA-B) that were selected for the fine typing strategy constitute the first ten markers of the set of 19 markers for the single pass classification into frequent and rare HLA alleles (HLA-A and HLA-B). Like with sequence-based HLA typing there are heterozygous combinations of HLA alleles that can not be resolved. However, there are fewer ambiguities with this method due to the mini-haplotypes that are provided.

20. Another object of the present invention is the use of said methodology of the invention is for screening of tissue donors, for example, bone marrow donors in registries for frequent and rare HLA types.

The description of the HLA alleles is based on the Anthony Nolan database (October 25 2003).

In addition to the aforementioned method, the invention includes yet other arrangements which will emerge from the description that follows, which refers to examples of supports according to the invention, as well as the annexed figures and tables, wherein:

Figure 1 describes 19 positions covered by mini-haplotyping assays for discrimination of HLA-A mapped onto the HLA-A allele A\*010101 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are

captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 2 describes 19 positions covered by mini-haplotyping assays for discrimination of HLA-B mapped onto the HLA-B allele B\*070201 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 3 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-DRB1 mapped onto the HLA-DRB1 allele DRB1\*034874 as reference. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

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Figure 4 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-A mapped onto the HLA-A allele A\*010101 as reference for the distinction of subgroups that can then be further analysed. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 5 describes 10 positions covered by mini-haplotyping assays for discrimination of HLA-B mapped onto the HLA-B allele B\*070201 as reference for the distinction of subgroups that can then be further analysed. Black boxes indicate an extension position while grey boxes indicate polymorphisms that are captured by the annealing of the respective primer of the primer pool. Pools are used in forward and reverse. Numbering is according to the transcription start of the cDNA.

Figure 6 describes Genotyping results of a CEPH family (1418, 01 = father, 02 = mother, 03 = child, 04 = child) for position HLA-B\_272. 1407,3 Da corresponds to

the addition of C to primer 6, 7, 8, or 9; 1422,3 Da corresponds to the addition of T to primer 6, 7, 8, or 9; 1431,4 Da/ 1430,9 Da corresponds to the addition of A to primer 6, 7, 8, or 9; and 1447,4 Da/ 1448,5 Da corresponds to the addition of G to primer 6, 7, 8, or 9.

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Table I represents HLA-A alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

Table II represents HLA-B alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

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Table III represents HLA-DRB1 alleles captured by the 10 markers in the different subgroups and additional positions that have to be typed to resolve the subgroups.

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Table IV represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-A (19 markers). The 10 markers required for the creation of subgroups are also contained. ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp means phosphorothioate group. The product analysed by mass spectrometry includes the base 5'of the most 5' sp.

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Table V represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-B (19 markers). The 10 markers required for the creation of subgroups are also contained. ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp means phosphorothioate group. The product analysed by mass spectrometry includes the base 5'of the most 5' sp.

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Table VI represents the list of the individual primers that are required to constitute the pools for mini-haplotyping of HLA-DRB1 (10 markers). ^ refers to the base used to attach the mass/charge tag, CT refers to the mass difference of the mass/charge tag, sp means phosphorothioate group. The product analysed by mass spectrometry includes the base 5' of the most 5' sp.

Table VII represents the resolution that can be generated with the 19 markers for the distinction of the frequent HLA alleles in HLA-A.

Table VIII represents the resolution that can be generated with the 19 markers for the distinction of the frequent HLA alleles in HLA-B.

Table IX represents the resolution that can be generated with the 10 markers for the distinction of the frequent HLA alleles in HLA-DRB1.

Table X represents the list of HLA-A alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

Table XI represents the list of HLA-B alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

Table XII represents the list of HLA-DRB1 alleles that are resolved with the 10 markers for the creation of subgroups. Each subgroup is separated by an empty line. Frequent alleles are shaded in darker grey, while lighter grey indicates the position that primers are extended onto.

### 25 Examples

Example: Mini-haplotyping at position 272 of HLA-B by the modified GOOD-Assay

A locus specific PCR product of exon 2 and exon 3 of HLA-B is amplified with a set of primers published by the International Histocompatibility Working Group, Technical Manuals (Hurly, Fernandes-Vina, Gao, Middleton, Noreen, Ren and Smith; www.ihwg.org/tmanual/Tmcontents.htm). The PCR product is incubated with SAP to remove all excess dNTPs. Then a single base primer extension at position 272 in the PCR amplicon is carried out. The set of primers, to generate the mini-haplotypes is

shown in Table V. Thereafter a 5'phosphodiesterase digest is applied to reduce the primers to a core sequence. After alkylation of the DNA backbone of the minihaplotype fragments the products are transferred onto a MALDI target pre-coated with matrix. Alternatively the matrix solution can be mixed with the samples and transferred onto the MALDI target to dry. The MALDI target is introduced into a MALDI mass spectrometer and analysed. The mass spectra show one or two mass peaks and that correspond to specific mini-haplotypes.

### PCR:

Forward primer, BAmp1 5'-G GGT CCC AGT TCT AAA GTC CCC ACG-3'(1.875 pmol), reverse primer, BAmp2 5'-CC ATC CCC GGC GAC CTA TAG GAG ATG-3' (1.875 pmol) an BAmp3 5'-AGG CCA TCC CGG CGG GCG ATC TAT-3' (1.875 pmol), 0.25 μl 10x PCR buffer (HiFi Platinum Taq) ), 0.3 μl MgSO<sub>4</sub> (50 mM), 0.2 μl of a mix of each dCTP, dATP, dGTP and dTTP (2 mM each), 0.25U engineered DNA polymerase (HiFi Platinum DNA Polymerase; Invitrogen) and 5 ng DNA fill to 3 ul with water. Cycling: 1. 94°C 3 min, 2. 94°C 20 sec, 3. 64°C 30 sec, 4. 72°C 30 sec, steps 2 to 4 are repeated 35 times, 5. 72°C 5 min.

## SAP digest:

20 1.75 μl of 50 mM Tris-HCl and 0.25 μl SAP (USB corporation, Cleveland, USA) are to add to the PCR product and this has to be incubated for 60 min at 37°C, followed by an incubation at 90°C for 10 min to denature the SAP enzyme.

## Single Base Primer Extension:

To the SAP treated PCR product 2 ul of an extension mix is to add. This mix contains 15 mM MgCl<sub>2</sub>, 0.1 mM of each of the four α-S-ddNTPs, 5 pmol of the extension primers set and 0,4 U of Thermosequenase. Cycling: 1. 94°C 2 min, 2. 94°C 15 sec, 3. 58°C 20 sec, 4. 72°C 20 sec, steps 2 to 4 are repeated 50 times.

## 30 PDE digest:

To the extension product has to be added 0.5 ul 0.5 M acetic acid and 1.5 ul PDE (5.1U) and incubate for at lease 120 min at 37 °C.

## Alkylation:

The alkylation is carried out by adding 21 µl of an alkylation mix and incubate for 15 min at 40°C. This mix contains 377 parts water free acetonitrile, 15 part 2M triethylamine/CO<sub>2</sub> (pH ~7.5), 75 parts 2mM Tris-HCl and 174 parts methyljodate.

5 The alkylation is to stop by adding 10 μl deionisated water. 5 μl of the resulted upper phase are to dilute in 10 μl 40% acetonitrile.

For MALDI target preparation and measurement with the MALDI mass spectrometer 0.5 μl of the final dilution are transferred onto a MALDI target pre-coated with matrix (α-cyano-4-hydroxycinnamic acid methyl ester). Measurement was carried out in a Bruker Autoflex with typically 18 kV acceleration voltage, pulsed ion extraction with a delay of 200 ns, and detection in linear detection mode. Results for CEPH family 1418 are shown in figure 6.

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## <u>Claims</u>

1. Method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) at a given position simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primers) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases.

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- 2. Method according to claim 1 where the DNA strand of step a) is produced by a DNA replication procedure such as PCR or rolling circle replication.
- 3. Method according to claim 1 where the combination of primers has slightly varying sequences so that all sequences of the haplotypes are represented by a perfectly matching primer.
  - 4. Method according to claim 3 where mass shifting tags are added to the individual primers sequences to make them uniquely distinguishable once the terminating base is added.
- 5. Method according to claim 1 where distinguishable termination products for known alleles are generated by extending the perfectly hybridised primer with a combination of dNTPs and ddNTPs or analogs thereof with a DNA polymerase to generate specific termination products.
  - 6. Method according to claim 1 where the GOOD assay is used.
- 25 7. Method according to any of the precedent claims where mass spectrometry, in particular MALDI or ESI mass spectrometry is used for analysis of the masses of products.
  - 8. Method for HLA typing according to any of the precedent claims above where set of multiple selected positions are queried to achieve sufficient information content.
  - 9. Method for HLA typing of HLA-A according to claims 1-8 where assays of the positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81, 268, 559, 92, 123 and 396 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.

10. Method for HLA typing of HLA-B according to claims 1-8 where assays of the positions: 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.

- 11. Method for HLA typing of HLA-DRB1 according to claims 1-8 where assays of the positions 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.
- 12. Method for HLA typing of HLA-A according to claims 1-8 where assays of the positions 98, 414, 539, 282, 571, 368, 256, 292, 238 and 270 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1) are used to generate subgroups A-O.
- 13. Method for HLA typing according to claim 12 where assays of the positions 224, 15 268, 376, 502, 561 and 616 are preferably analysed to resolve subgroup HLA-A A; positions 126 and 526 to resolve subgroup HLA-A B; positions 81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420, 427, 453, 485, 489 and 502 to resolve subgroup HLA-A\_C; positions 160, 200, 362 and 524 to resolve subgroup HLA-A D; positions 180, 299, 301, 302, 346, 418, 453, 517, 524, 526, 527, 557, 559 and 560 to resolve subgroup HLA-A E; positions 299, 301, 302, 341 and 583 to 20 resolve subgroup HLA-A F; positions 127, 341, 399, 480, 502, 503, 524, 526, 527, 553, 559, 560 and 565 to resolve subgroup HLA-A\_G; positions 228, 233, 463, 519, 530 and 583 to resolve subgroup HLA-A H; positions 102, 275, 317, 362, 418, 419, 497, 524, 555, 595 and 618 to resolve subgroup HLA-A\_I; 25 positions 92, 331, 453, 524, 559, 560 and 564 to resolve subgroup HLA-A J; positions 78, 81, 123, 125, 142, 144, 194, 268, 294, 324, 355, 362, 396, 403, 419, 453, 456, 477, 493, 517, 524, 526, 527, 559 and 560 to resolve subgroup HLA-A\_K; positions 113, 299, 301, 302, 308, 311, 523, 524 to resolve subgroup HLA-A\_L; positions 171, 363, 498 and 559 to resolve subgroup HLA-A M; positions 30 376, 426, 527, 555, 557 and 595 to resolve subgroup HLA-A N; position 299 to resolve subgroup HLA-A O are used.
  - 14. Method for HLA typing of HLA-B according to claims 1-8 where assays of the positions 539, 419, 559, 412, 272, 362, 302, 363, 206 and 369 (according to the

numbering of the HLA-B gene starting at DNA sequence position 1 of exon 1) are used to generate subgroups A-AC.

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- 15. Method for HLA typing according to claim 14 where assays of the positions 259, 341 and 473 are preferably analyzed to resolve subgroup HLA-B A; positions 106, 144, 222, 259, 273, 311, 313, 418, 445, 493, 528 and 540 to resolve subgroup HLA-B B; positions 319, 416, 545 and 572 to resolve subgroup HLA-B C; positions 106, 131, 165, 215, 243, 277, 292, 322, 481, 582, 603 and 616 to resolve subgroup HLA-B\_D; positions 106, 146, 165, 181, 238, 259, 263, 292, 328.1/329, 379, 435, 453, 463, 485, 526, 571, 572 and 583 to resolve subgroup HLA-B E; positions 142, 171, 255, 257, 395, 430, 544, 566 and 572 to resolve subgroup HLA-B\_F; positions 117, 247, 248, 277, 345, 418, 489 and 527 to resolve subgroup HLA-B\_G; positions 134, 141, 200, 213, 259, 304 and 527 to resolve subgroup HLA-B\_H; positions 83, 141, 211, 222, 242, 322, 404, 414, 435, 463, 502, 527, 544, 571, 572 and 583 to resolve subgroup HLA-B\_I; positions 103, 142, 222, 243, 259, 292, 477, 486 and 499 to resolve subgroup HLA-B J: positions 103, 259, 292, 295, 527 and 583 to resolve subgroup HLA-B\_K; positions 320 and 500 to resolve subgroup HLA-B\_L; positions 311, 527 and 583 to resolve subgroup HLA-B\_M; positions 119, 292, 259, 319, 425, 527, 546 and 583 to resolve subgroup HLA-B\_N; positions 97, 142, 245 and 527 to resolve subgroup HLA-B\_O; positions 97 and 175 to resolve subgroup HLA-B P; positions 246 and 277 to resolve subgroup HLA-B\_Q; positions 246, 292, 311 and 503 to resolve subgroup HLA-B\_R; positions 103, 261, 309, 311 and 474 to resolve subgroup HLA-B\_S; positions 97, 103, 106, 243, 259, 292, 404 and 524 to resolve subgroup HLA-B\_T; positions 259 and 320 to resolve subgroup HLA-B U; position 106 to resolve HLA-B\_V; positions 97 to resolve HLA-B\_W; positions 97, 106, 257, 418 and 463 to resolve HLA-B\_X; position 106 to resolve HLA-B\_Y; positions 106 and 144 to resolve HLA-B\_Z; positions 117, 247, 248, 283, 345, 418, 489, and 527 to resolve HLA-B\_AA; positions 106 to resolve HLA-B AB; positions 548 to resolve HLA-B AC.
- 30 16. Method of HLA typing according to claim 11 to resolve subgroups A-P of HLA-DRB1.
  - 17. Method for HLA typing according to claim 16 where assays of the positions 123, 174, 250, 278 and 317 are analysed to resolve subgroup HLA-DRB1\_A; positions 192, 203, 256 and 259 to resolve subgroup HLA-DRB1\_B; 256, 260, 317 and 351

to resolve subgroup HLA-DRB1\_C; positions 155, 204, 233, 239, 256, 304, 357 and 366 to resolve subgroup HLA-DRB1\_D; positions 122, 171, 257 and 317 to resolve subgroup HLA-DRB1\_E; positions 164, 167, 171, 230, 235, 306, 317, 321 and 337 to resolve subgroup HLA-DRB1\_F; positions 164, 257, 266 and 303 to resolve subgroup HLA-DRB1\_G; positions 164, 181, 188, 220, 229, 256, 266, 317 and 318 to resolve subgroup HLA-DRB1\_H; position 257 to resolve subgroup HLA-DRB1\_I; positions 181, 239 and 357 to resolve subgroup HLA-DRB1\_J; positions 122, 144, 239, 303, 317, 318 and 321 to resolve subgroup HLA-DRB1\_K; positions 118, 161, 257, 260, 318 and 321 to resolve subgroup HLA-DRB1\_L; positions 165, 257, 293 and 303 to resolve subgroup HLA-DRB1\_M; positions 177, 240, 256, 257 and 357 to resolve subgroup HLA-DRB1\_N; positions 150 175, 230, 236 and 321 to resolve subgroup HLA-DRB1\_O; positions 115, 220 and 317 to resolve subgroup HLA-DRB1\_O; positions 115, 220 and 317 to resolve subgroup HLA-DRB1\_P are used.

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- 18. Kit for the implementation of the procedure according to claims 1 17 comprising pools of primers.
  - 19. Use of the method according to claims 1-17 for screening of tissue donors.
  - 20. Use according to claim 19 for bone marrow donors in registries for screening of frequent and rare HLA types.
- 20 21. Use of the primers represented in Table IV, V and VI to carry out HLA typing.

## **Abstract**

## Method for HLA typing

A method for the identification of DNA sequence elements in complex and highly variable sequences is described. The method consists of identifying a short sequence element of several DNA bases (2-6 bases) at a given position in the genome simultaneously on all parental alleles. The method allows differentiating mini-haplotypes on different alleles in one analysis. The method consists of carrying out an enzymatic primer extension reaction with a combination of extension primers (pool of primers) and analysing the products by mass spectrometry. The pool of primers is assembled in such a way that the primer extension product allows unambiguous identification of both the primer of the pool that was extended and the base that was added. The method is of great utility for DNA sequences harbouring many SNPs close to each other with many possible haplotypes. Such sequences are known in the Major Histocompatibility Complex (MHC). This method is particularly well suited for DNA-based HLA typing and in combination with a suitable selection of sites tested, it is superior in ease of operation to conventional HLA typing methods. We have identified sets of these assays for HLA-A, HLA-B, and HLA-DRB1 that allow unambiguous four-digit HLA of each of these genes with between 11 and 28 queried markers.

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\*123 ATCCGTGTCCCGGCCCGGCG<mark>GGMM</mark>AGCCCCGCTTCATCGCGGTCTACGTGGACGACGAGATCGTGCGGTTCGACAGCGACGCCGGGGGCCAGAAGATGGAGCCGCGGG 

\*238 \*256 268\* \*270 282\* 292\* CGCCGTGGATAGAGCAGGAGGGAGGTATTGGGAC<mark>ORGR</mark>AGACA<mark>RTG</mark>AAGGQCCA<mark>G</mark>TCACA<mark>GRATG</mark>ACCGAGGGAACCTGGGGGACCCTGCGGGGCTACTACAACCA

368\* GACCGCGGGGTCGGGGCCAGGTTCTCACATCCAGATAA<mark>TGWA</mark>TGGCTGCGACGTGGGGCCGGACGGCG<mark>GWTW</mark>GTCCGCGGGTA<mark>WAWG</mark>CAGGACGCCTACGAAGGAT

\*559 A*GGGC<mark>©GT</mark>IGCGTI<mark>GGAWG</mark>GGCTCCGCAGATACCTGGAGAAGGAGAGGAGGGGCGACGGGGTA*CCAGGGGGCCACGGGGGCGCCTCCCTGATCGCCTATAGATCTCCCGGGC

TGGCCTCCCAC TO THE TOTAL 
\*123 ATCCGTGTCCCGGCCGGCGGGGGGTTCATCGCCGTGGGCTACGTGGACACACGCAGTTCGTGCGGTTCGACAGCGACGCCGCGAAGAAGATGAAGCGCGGGG  368\* GACCGC*GGGGTCGGGGTTCTCACACCATCCAGATAA<mark>TGMA</mark>TGGCTGCGACGTGGGGCCGGACGGGGGMA<mark>GMG</mark>TCGCGGGTA<mark>GMG</mark>CAGGACGCCTACGACGCAAGGAT* 

\* 453 TACATCGCCCTGAA<mark>GGAG</mark>GACCTGCGCTCTTGGACGCGGGGGGGGGGGGGGGTCCAGATCACCA<mark>AGGCG</mark>CAAGTGGGAGGCGGTCC<mark>ATGG</mark>GGCGGAGA<mark>AGAG</mark>GAGAGTCTACCTGG

\*559 571\* AGGGC<u>GGGT</u>GCGT<u>GGAGG</u>GGCTCCGCAGATACCTGGAGAAGGAAGGAGACGCTGCACGGGTACCAGGGGCCACGGGGCGCCTCCTGATCGCCTATAGATCTCCCGGGC

TGGCCTCCCAC

FIGURE 2

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CTAGAGAAGCCAATCAGCGTCGCCG

crectectc

CGCAGGTCACGACTCCCCCATACGGCCCGGGTCGCCCGAGTCTCCCGGGTCCGAGATCCGCCTCCCTGAGGCCGGGGACCCGCCAAGACCTCGACCGGCGAGAGCC

\*435 ACCCTd<u>CA短角GOA開</u>GTA<mark>GGG</mark>TGCGACGGGGCGGGGGGGGGGGGGGGG<mark>超A開G</mark>A<mark>CCAM</mark>GACCGTACGACGGCAA<mark>C</mark>算A可TACATCGCCCTGAACGACCTGCGCT 412\* 418\*\*419 \*369 162\*\*363

527\* \*539\* \*559 \*571 CCTGGACCGCGGACACGGGGTCAGTCACCCAGGGCAAGTGGGAGGCGGCCG<mark>MTGA</mark>GGCGGAGG<mark>AGGG</mark>GAGAGCCTACCTGGAGGGC<mark>GMGT</mark>GCGTGGAGTMGGGTCCGCAG

4/6

CTAGAGAAGCCAATCAGCGTCGCCG 

CGCAGGTCACGACTCCCCCACGTACGGCCCCGGGTCGCCCGAGTCTCCGGGTCCGAGATCCGCCTCCTGAGGCCGGGGACCCGCCCAGACCCTCGACCGGCGAGAGCC

362\*\*363 \*369 ACCCTC<u>CA®MGTAGGG</u>TGCGACGGGCGCGGGGGCGCCCTCCTCCGGGMAMGACCAGTAMGCGTACGACGCCAAGGATTACATCGCCCTGAACGAGCACCTGCGCT

539\* CCTGGACCGCCGCACACGCGCTCAGATCACCCCAGCGCAAGTGGGAGGCGGCCGTGAGGCGGAGGAGGGGAGGCCTACCTGGAGGGGG<mark>AGT</mark>GCGTGGAGTGGCTCCGCAG

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\*125 GAACA<mark>的高限的影響的影響的影響的影響。</mark>rescaecttaagt<mark>nga</mark>arescattetetetetetetetetetetetetetetetaaagatetaataateaagaag<mark>aagute</mark>te

227\* \*308 GCTTCGACACGTGGGGGAATA

MANAGEMENT CON CONTRACTOR CONTRAC 341\* \*345 CTACTGCAGACACAACTACG<mark>GGG<mark>H</mark>TGG<mark>HGAG</mark></mark> \*345

# IGURE 5

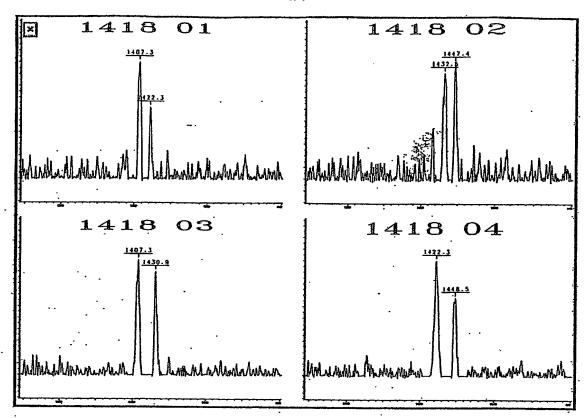


FIGURE 6